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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 255.
B. T. GALLOWAY, *Chief of Bureau.*

THE STRUCTURE AND DEVELOPMENT OF CROWN GALL:

A PLANT CANCER.

BY

ERWIN F. SMITH,
Pathologist in Charge of Laboratory of Plant Pathology,
AND
NELLIE A. BROWN AND LUCIA McCULLOCH,
Scientific Assistants.

ISSUED JUNE 29, 1912.

WASHINGTON:
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BUREAU OF PLANT INDUSTRY.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., May 9, 1912.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 255 of the special series of this bureau the accompanying technical paper by Dr. Erwin F. Smith, Miss Nellie A. Brown, and Miss Lucia McCulloch, entitled "The Structure and Development of Crown Gall: A Plant Cancer."

This paper is the result of many months of critical study of hundreds of serial sections prepared on the microtome; and so far as relates to the photographic demonstration of the presence of the causal organism within the proliferating cells, to several years of laborious and discouraging experimentation with a variety of fixing agents and stains. Only recently has it been possible to demonstrate clearly by means of the microscope the presence of the parasitic organism within the cells, although the authors have known for more than five years that this organism *must be* located within the cells, because in galls shown by the agar poured-plate method to contain the bacteria, no granules of any sort occur between the cells or in the lumen of the vessels.

Had Dr. Smith's researches on crown gall been confined only to morphology (excluding parasitology) it would be reasonable for him to make precisely the same statement now commonly made by research workers in cancer, viz, "The cell itself is the only parasite. That fully explains all the observed phenomena." But, adding the bacteriological evidence, we see for the first time clearly that while it is the rapidly proliferating cancer cells that do the mischief they are impelled to behave in this way only because they are under the stimulus of a foreign organism which does not destroy them, but irritates them to rapid division and passes over into certain of the daughter cells to repeat the process indefinitely. This, it can not be denied, is a discovery of the first magnitude in pathology.

A preliminary announcement of certain of these new discoveries was made a year ago, to wit, the existence of a tumor strand and of stem structure in secondary tumors. The paper herewith submitted

furnishes the promised photomicrographic proofs in support of those statements.

The interest which the preliminary statements have awakened, not only among plant pathologists, but also among medical men in all parts of the world, and the manifestly important bearing of these researches on the origin of malignant human and animal tumors, make it desirable to publish the investigations in full. It is recommended, therefore, that the paper be published as submitted, with all of its illustrations, and at as early a date as possible. The illustrations submitted are the essential part of the paper, the text, for the most part, being only a running commentary.

Respectfully,

B. T. GALLOWAY, *Chief of Bureau.*

Hon. JAMES WILSON,
Secretary of Agriculture.

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THE STRUCTURE AND DEVELOPMENT OF CROWN GALL: A PLANT CANCER.

INTRODUCTION.

This is a bulletin on the histology of crown gall. For five years the senior writer has been hammering away at the idea that crown gall of plants resembles malignant human tumors and can be made to throw a flood of light on the origin of the latter, which is still shrouded in obscurity and believed by the majority of pathologists to be of nonparasitic origin (vide Bashford, Reports of Imp. Cancer Research Fund).

A year ago the discovery of a tumor strand and of a stem structure in secondary tumors in leaves gave a strong impetus to this view. In the interim this contention has been expressed publicly several times (vide Science, Feb. 2, 1912, and Centralb. f. Bakt. 2te Abt., 1912). The bulletin here offered is in the nature of supporting evidence.

This view received only a cool welcome at first, very likely through more or less inapt presentation, but recently it has received respectful attention. In October, 1910 (Int. Cancer Congress, Paris), Jensen, of Copenhagen, expressed similar views respecting a tumor on the sugar beet.

In the meanwhile, on the animal side several publications of prime importance, all within the year, or very nearly, have tended strongly to unsettle the crystallizing belief in the nonparasitic origin of cancer. These have been as follows:

(1) The announcement by Peyton Rous (Jan. 21, 1911) that a chicken sarcoma is inoculable in the absence of living chicken cells, i. e., with fluid freed from the ground sarcoma by centrifuging, and also by filtration through moderately coarse Berkefeld bougies. Fine bougies will not serve. Later, in the Journal of Experimental Medicine, he furnished what seem ample proofs of this contention. Very recently he has shown that tumor material dried for six months is still infectious. (April 4, 1912, An. Meeting Am. Asso. for Cancer Research.)

(2) The discovery by von Dungern that when a round-celled sarcoma of the dog was grafted on the fox *only fox cells grew.* (Muenchner Med. Wochenschrift, Jan. 30, 1912, p. 238.)

(3) The recent statements by Wassermann, Keyser and Wassermann, that cancer cells of mice (both carcinoma and sarcoma) have a selective affinity for salts of selenium when these are passed into the blood stream in combination with eosine, thus showing that the contents of tumor cells is chemically distinct from that of normal cells. (Deutsche Med. Wochenschrift, No. 51, Dec. 21, 1911, p. 2389).

CROWN GALL A NEOPLASM.

That we have in crown gall peculiarities of neoplastic growth which remove it from all ordinary plant diseases and place it in the category of the true tumors (atypical blastomas) is the burden of this bulletin.

The phenomena of growth in this disease are in the highest degree surprising and are quite unlike anything hitherto known in plant pathology. It is believed for reasons that will become evident further on that for comparable phenomena we must turn to animal pathology, and particularly to that part of it which deals with malignant tumors. Among the latter only do we find growths which appear to be identical. In other words, the contention of this bulletin is that crown galls are to all intents and purposes cancers.

The histological evidence on which this statement is based is presented in the following pages in the form of photomicrographs, only so much text being appended as shall serve to make the sun pictures intelligible. Accompanying these photomicrographs are photographs of the inoculated plants, introduced to show relation of parts. It is believed that more can be learned as to the nature of this disease from an inspection of these pictures than from any number of pages of text, because texts and even drawings are liable to be colored more or less by the beliefs of the writer, whereas the camera reproduces only what is present, albeit sometimes rather imperfectly.

The morphological likeness of crown gall to malignant animal tumors consists in—

(1) A peripheral growth of tumor cells out of preexisting tumor cells, with absence of any capsule or well-defined limit to growth. The growth is injurious and extraphysiological, and, exactly as in human cancer, the cell itself is the only visible parasite.[1]

---

[1] "We can say, then, that cancer is not due to a specific parasite or parasites, but, on the other hand, we can say that cancer cells themselves act as parasites. This view will explain all the phenomena of cancer."—*Dr. Charles Powell White.*

"The whole basis, objective and theoretical, of the cancer parasite has been traversed again and again, with the uniform conclusion of those who finish the journey that the cancer parasite is the cancer cell."—*Dr. James Ewing, in his Cancer Problems.*

"*Cancer bodies.*—There exists, in fact, a very remarkable series of localized degenerative changes in cancer cells that have been the cause of active controversy for now close upon 20 years; nor can it be said that the controversy is as yet at an end, although the main body of pathologists of all countries is now of the opinion that these appearances are degenerative and not parasitic. For some years, however, the parasitic theory of cancer had active and enthusiastic supporters."—*Adami, Principles of Pathology, vol. 1.*

(2) The existence of a well-developed supporting stroma.

(3) The formation of tumor strands which extend from the primary tumor in various directions.

(4) The development on these tumor strands of secondary tumors which have the structure of the primary tumor even when they are located in other organs.

(5) The existence of giant cells, i. e., cells which contain several nuclei, and of rapidly proliferating anaplastic cells.

(6) The occurrence of many amitotic nuclear divisions and of occasional abnormal mitotic divisions, i. e., divisions in which more chromosomes pass to one pole than to the other.

TUMOR CELLS FROM TUMOR CELLS.

A study of the growth of the crown gall shows that certain cells which have received the initial stimulus (infected needle pricks in our experiments) divide repeatedly and often very rapidly (Bul. 213), giving rise to a mass of soft tissue which is not inclosed in a capsule but grows peripherally, infected mother cells giving rise to daughter cells, and so on, indefinitely. These cells also stimulate other uninfected cells into rapid growth. Unlike the reparation of a wound, the growth is not limited to the physiological needs of the plant, but continues removed from the control of the plant except in so far as it is dependent on the latter for its food and water supply.

Apparently any meristematic cell may originate such tumors, but if they are not provided with a stroma they remain small and soon perish. This conclusion is based on a series of shallow versus deep inoculations into daisy stems. When the needle punctures were only 0.5 mm. deep, i. e., only into the region of the cork cambium, a stroma appeared, but the nodules remained small, and rotted away within a few weeks. When the needle pricks were 1 mm. or more in depth, i. e., when they entered the cambium region of the stem, much larger and longer-lived tumors resulted and these were abundantly vascularized.

GIANT CELLS.

Multinucleate cells occur which are perhaps comparable to the giant cells of the animal histologist. Cancer specialists have divided these into two groups, viz, foreign-body giant cells in which the stimulus is some introduced foreign substance, and genuine ones in which no foreign bodies are visible. There is probably no real distinction other than that those occupied by parasites are malignant and those induced by non-living granules are harmless. The cells in question in crown gall are not very large, but they contain several nuclei. Four nuclei in one cell is the most we have seen, but it is probable that larger numbers occur.

It would seem from our studies, which, however, are incomplete, that most of the cell divisions in crown gall are by mitosis. Frequently, however, we have found nuclei variously lobed and in process of amitotic division, and this is probably the way in which several nuclei are formed in one cell. (See fig. 1 and top figure of Pl. CVIII.) The whole subject of the cell mechanics of the tumor is reserved for further study.

THE STROMA.

Pari passu with the growth of the tumor cells new supporting tissues are developed in the tumor in various places. These supporting

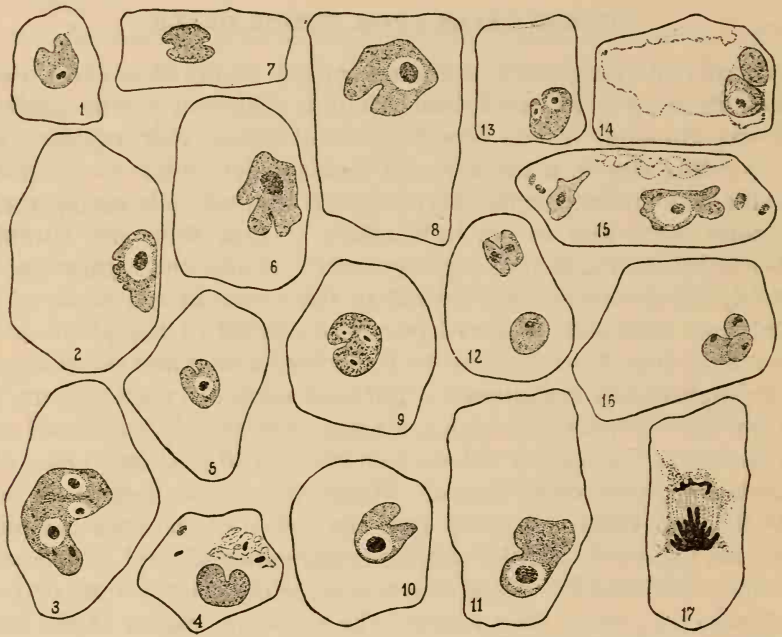


FIG. 1.—Nuclear divisions in crown gall: Nos. 1 to 16, cells showing stages of amitotic division; No. 17, mitotic division in which more chromosomes have passed to one pole than to the other. Material fixed in Flemming, and stained with Heidenhain's iron hæmatoxylin.

tissues consist of pitted vessels and wood fibers, but frequently the latter are scanty or absent. Sieve tubes are also present and are conspicuous in the outer part of secondary tumors. Spiral vessels are sometimes present in the tumor tissue, but never as *new growths*. They occur only as fragments ruptured from their normal position and carried away by the overwhelming growth of the tumor tissue.

A most interesting question arises here: Does the stroma originate in the tumor, or is it a growth from the surrounding tissues? Studying the origin of the pitted vessels in secondary tumors it seemed at first as if they must be derived from the already existing leaf trace

stimulated into abnormal development by the presence of the tumor strand, but such is not usually the case. They are developed in most instances, so far as we have been able to determine, out of the tumor strand itself. Wood fibers when they are present, and likewise the sieve tubes, originate in the same way. This should not seem strange, since the tumor strand is an actively growing meristematic tissue. In some cases, however, the extreme edges of the cambium of the leaf trace seem to proliferate tracheids, which enter the tumor. Further studies will be made.

That the pitted vessels of the stroma are *new growths* admits of no doubt whatever. Their number in secondary leaf tumors far exceeds the normal number in the leaf trace, often as much as 100 to 1, and in early stages of development, such as that shown on Plate XLVIII, we have succeeded in tracing the abnormal vessels into the tumor strand, finding tracheids on one side of the abnormal meristem and sieve tubes on the other side, the tissues of the leaf trace being either uninvolved or only slightly affected. The pitted vessels found in the tumor serve to furnish it with water for its growth. This growth, when the stroma is abundant, forms a very hard, slow growing, fibrous, and resistant mass. But often the galls are soft and much exposed to decay, and in such cases the stroma is scanty and the woody part of it composed only of scattering pitted vessels unsupported by wood fibers. There are all sorts of transitions between these two conditions.

THE TUMOR STRAND.

Soon after the appearance of a primary tumor, particularly if the plant is well nourished and growing rapidly, tumor strands push out of it into the normal tissues, generally, it would seem, along lines of least resistance. These, of course, are invisible externally, but may be found by dissecting the basal parts of the tumor, and if of any size they are readily detected without the use of the microscope, i. e., by their peculiar structure and color (daisy). Subsequently when they are extending in thin leaves they may be recognized sometimes by a slight tumefaction on the midrib or leaf vein, which ceases beyond the advancing tip of the strand; and especially by the development on the strand of secondary tumors hidden at first by the overlying normal tissues of the leaf but soon giving to the latter a puffed-up appearance and subsequently coming to the surface of the plant by crushing and rupture of the overlying parts. Secondary tumors are a very common phenomenon in the Paris daisy and always, so far as observed, they are outgrowths from the deep-lying tumor strand, which is itself an outgrowth of the primary tumor.

This tumor strand in the Paris daisy makes its way exclusively, so far as observed, in the protoxylem region of the plant, i. e., in the

region of the primitive spiral vessels, where it is often under great pressure, especially in the stem. (Pl. LXII.) So far as known, the tissues under pressure are not absorbed, but they are often flattened, crushed, split open, and exfoliated by the growing tumor. In a tobacco stem a tumor strand was observed in the bark parenchyma. In a number of instances we have found short tracheids in process of development directly from cells of the tumor strands, their lignification being yet incomplete and their nuclei still present. (Pl. LXVIII.)

STRUCTURE OF SECONDARY TUMORS.

If this disease were a granuloma we should expect the secondary growths to take on the structure of the organ in which they are located but such is not always the case.

When primary tumors develop in the top of beet roots, the secondary tumors in the midrib of the leaf have the many-ringed structure of the root. Usually when primary tumors develop on the soft stem of a Paris daisy secondary tumors appear in the leaves after a few weeks, and these tumors have a distinct *stem structure*—a structure which is not that of the leaf but of an invading destructive growth. This growth appears in one or more of the leaf traces, first as a tumor strand. This strand proliferates a variety of tissues—pitted vessels, sieve tubes, wood fibers (?), and medullary rays. These new vessels make the stroma of the tumor which, by fusing with the leaf trace, causes that part of the leaf to assume the form of an imperfect, rather fleshy, perishable *stem*, the tumor strand occupying the center.

This is such a peculiar phenomenon and so unlike anything hitherto known in plant diseases that the reader might well be excused for scepticism; the statement, however, is well supported by many observations and admits of no doubt, as may be seen from the accompanying photomicrographs.

ETIOLOGY OF THE TUMOR.

The cause of this disease is a schizomycete, *Bacterium tumefaciens* Smith and Townsend. The proofs of this statement, together with the morphological and cultural characters of the organism were given *in extenso* in Bulletin 213, Bureau of Plant Industry, and will not be rehearsed here.[1] Earlier papers also may be consulted. The organism was described and named after careful determination of its pathogenic properties April 26, 1907 (vide Science, n. s., vol. 25, p. 672, and Centralb. f. Bakt., 2 Abt., XX Bd., p. 89). Following the chart of the Society of American Bacteriologists, the group number of this organism is 212.2322023.

[1] Copies of Bulletin 213, Bureau of Plant Industry, U. S. Dept. of Agriculture, issued Feb. 28, 1911, may be had from the Superintendent of Documents, Government Printing Office, Washington, D. C. Price 40 cents. Add 10 cents for postage to foreign parts.

LOCATION OF THE BACTERIA.

The bacteria causing this neoplasm are located inside the cells, and it is the stimulus of their presence which causes the cell to divide abnormally by throwing it out of balance. Probably this stimulus also extends to many surrounding uninfected cells.

Since we have known the peculiarities of our organism it has been possible to prove, by means of agar poured plates, that the bacteria occur not only in the primary tumor but also in the secondary tumor and in the connecting tumor strand. By means of subcultures from single bacterial colonies we have produced the tumor hundreds of times (Bul. 213, p. 133), but it has not been easy to demonstrate the bacteria microscopically in the tissues. In most plant diseases of bacterial origin hitherto investigated (and the senior writer has been engaged in their study for more than 19 years) the demonstration of the bacteria in the tissues is a comparatively simple affair for any one possessed of fairly good technique. Not so here. We have believed for a long time that the locus of the bacteria must be the interior of the proliferating cells, because high powers of the microscope show that in rapidly proliferating tissues exactly like those known by the poured-plate method to contain the organism there are no granules (bacterial or other), either in the intercellular spaces or in the vessels, except sometimes near the entrance of the needle. Now if an organism is known to be present in one of three places and is not present in two of them, it must occur in the third. Both by diffusion from thin sections and by poured plates we have proved the bacteria to occur in the tumor, and if they do not occur between the cells or in the vessels, and the microscope shows that they do not, then *they must occur* within the cells. There is no other alternative unless we suppose them ultramicroscopic, i. e., totally unlike their form on culture-media, and also unlike the rods and Y-shaped bodies that diffuse out of the sections. (Fig. 2.) Then, of course, they might occur anywhere. Moreover, for such proliferation phenomena as that here described, extending as it does in a narrow cord of cells long distances from the primary tumor, it appears to be absolutely essential to the understanding of the mechanism of the controlling cell divisions that the stimulus should come from within the cell.[1] And that it does come from within admits of no doubt. First, as we have said, because there are no bacteria in the vessels or spaces between the cells; second, because we have occasionally seen rod-shaped, jointed, bacteria-like bodies moving about slowly within the living cells; third, because in a few instances we have been able to see them sharply delimited in

[1] It is inconceivable to the writer that a foreign organism, by any localized and brief presence in the tissues, should so modify cell inheritance that, after the organism and its products have disappeared, the cells should continue to develop abnormally rather than return to their normal habit.

stained preparations (Pls. CV, CVI, and CVII), and have not been able to find similar bodies in rapidly growing healthy tissues treated in the same way; and fourth, because the bacteria occur in the tumor strand and in the secondary tumor (evidence of colonies on poured plates and successful inoculations with subcultures therefrom).

The difficulty of demonstrating bacteria within the cell lies in the fact that commonly they are not very numerous and are mixed in with various cell inclusions which also stain with all the common bacterial stains, and some of which interfere with their satisfactory identification. No one who has not investigated the minute structure of the cell under high powers of the microscope knows the difficulties which lie in the way. The organism stains readily when taken from cultures, but practically all of our attempts to differentiate it in thin sections using the common basic anilin stains have been failures.

As in leprosy, these bacteria are in the protoplasm of the cell, *but not within the nucleus.* This seems to be made out with sufficient certainty by means of gold chloride impregnations which have given beautifully clear pictures, i. e., a farrago of granules (gold precipitates) and small groups of rod-shaped bodies resembling bacteria (some in division) stained deeply in the protoplasmic sac with the nucleus absolutely free and pale almost to invisibility. The protoplasm of the cell outside of the nucleus likewise remains colorless. By counter-staining with eosine the protoplasmic masses and the cell walls also become well defined. With the substage diaphragm wide open the granules are brown dots of various sizes, while the bacteria are deep blue-black rods, often crooked, or in clusters, pairs, chains, short filaments, or involution forms such as occur in culture media. When the diaphragm is closed the granules become paler (a gold color), but the bacteria retain their deep blue-black color. Sixty such rods were counted in the field from which figure 1 of Plate CV was photographed.

The group of bacteria shown in figure 1 is in the interior of a section 15μ thick. Figure at right is from same spot as 1, at a different focus. It shows 8 bacteria, 4 out of focus. As many as 100 bacteria have been seen within a single cell, all beautifully sharp (vide Plate CVI), but usually we found a lesser number and frequently none whatever, as though only a portion of the cells were occupied by them. Indeed, it would seem from our preliminary studies as though less than 1 per cent of the cells of the tumor are actually occupied by the bacteria. We hope to discuss this phase of the subject more fully in a subsequent bulletin.

By nitrate of silver impregnations we have also stained bodies within the cell which appear to be bacteria, and occasional cells show these in large numbers, but most of them only sparingly. In general, this method, which we tried first, seems less good than the gold chloride method.

The best preparation yet obtained for showing cell inclusions, which are not bacteria but might easily be mistaken for bacteria and were so mistaken by us at first, is a very thin section (5μ) stained by Gram but washed in amyl alcohol. This leaves the violet-blue stain in many kinds of bacteria from which the ethyl alcohol of the true Gram's stain removes it, *Bact. tumefaciens* in cultures being one of these. It was therefore thought to be well adapted for demonstrating them in the tissues. The material from which this section came was fixed in a moderately strong Flemming. The section was stained deeply and washed until very little color remained in the tissues. Most or all of the stained bodies at first suspected of being bacteria are probably lenticular chloroplasts seen on edge. These divide in the same way as bacteria, but they are not rounded at the ends. They can be distinguished from the latter also by the fact that they are not clustered and are circular and pale when not seen edge on. In the sections they show when seen on edge the same intensity and hue of color as the nucleus. These bodies are shown in Plates CVIII and CIX. Many times at least they appear to lie embedded in the wall of the protoplasmic sac (Pl. CIX). Sections stained with Heidenhain's iron haematoxylin also show these bodies very well.

One of the best evidences of the occurrence of the bacteria in the cells is the demonstration of Y-bodies, since there is nothing likely to assume this form in the normal cells of the plant. In Bulletin 213 (p. 107) we figured and described Y-shaped bodies which were obtained from cultures of *Bact. tumefaciens*. These occur in cultures exposed to low temperatures, to sodium chloride, to acetic acid, or to the by-products of the growth of the organism in the presence of peptone and sugar. They are fairly common in old cultures and are not unlike those assumed by many other bacteria when subjected to adverse conditions, e. g., bacteria occurring in the root nodules of legumes. At that time we believed them to occur also in the plant and to be responsible for the slow initial development of the poured-plate colonies (Bul. 213, p. 168), but had not found them. We have now found them *in situ* in the cells of the tumor (Pls. CV, CVI, and CVII) and have obtained them both from the sugar beet and from the daisy by allowing thin sections of fresh tumors to diffuse in water (double distilled from glass) on sterile slides (fig. 2). There is no doubt whatever as to their occurrence in the cells of the tumor, and we now have a sufficient and satisfactory explanation for the often observed slow development of the bacterial colonies on plates inoculated directly from the tumor (vide Bul. 213, pp. 22, 108, 168), because it is a well-known fact that involution forms are either dead or so reduced in vitality as to be slow to resuscitate. Those we obtained by adding dilute acetic acid to young agar and bouillon cultures were

either dead when tested on agar poured plates or developed colonies slowly.

TWO HYPOTHESES.

I. *Hard versus soft galls.*—Whether a crown gall shall develop as a hard gall or a soft gall would seem to depend chiefly if not altogether on which meristem cells receive the initial impulse. If the cells first

FIGURE 2.—Free-hand drawings of rods and involution forms of Bacterium tumefaciens made in 1911 from young tumors, the slides being obtained by allowing sections of the galls to diffuse for an hour in distilled sterile water, after which they were removed, the fluid on the slides evaporated, and the residue stained 20 to 30 minutes in a dilute solution of basic fuchsin. The surface of the gall was removed with red-hot knives before making the sections, and all of the instruments and fluids were free from bacteria: *A*. Daisy on daisy, May 25 (Brown). About 1 sq. cm. examined in 2 hours; *B*. Daisy on daisy, May 23 (Brown), 2 sq. cm. of slide examined during 5 hours, several other Y's seen; *C*. Hop on red table beet, May 20 (Brown); *D*. Hop on red table beet, May 19 (Brown); *E*. Hop on sugar beet, May 18 (Smith). The rods were about 1.5 to 2 μ x 0.5 to 0.7 μ. There was not more than one distinct one per field (2 mm. Zeiss 1.30 n. a. oil im. obj. and No. 8 comp. oc.). Frequently one of the two segments of a pair was swollen. More than twice as many involution forms were seen as drawn. There was about one to every 8 or 10 fields. About 10 sq. cm. of the slide were examined. *F*. Hop on sugar beet, slide XXX, May 19 (Smith). Another part of *E*. Eight Y's not drawn. All well stained with diaphragm wide open; *a* measured 1.3 x 0.8 μ; *b*, 3 x 0.5μ, the latter was by itself, i. e., free from other granules with perfectly sharp margins and a deep stain.

infected are principally the mother cells of medullary rays, we may assume that the gall will be a "soft gall," and readily inclined to decay. If, on the contrary, the needle or other carrier of infection wounds principally those meristem cells which give rise to tracheids and wood fibers, the gall will be a "hard gall," of slow growth and long

duration. This hypothesis would seem to account for all the histological differences observed in crown galls and for at least a part of their recognized gradations in virulence.

II. *Crown gall a symbiosis.*—The relation between host and parasite in this disease may be regarded as a symbiosis in which the bacterium has the advantage. It derives its food from the cells of the host, and drives them at a breakneck speed. It gives to them in return its waste carbon dioxide for the use of their chloroplasts. It does not destroy the cells of the host, but only stimulates them into an abnormal and often exceedingly rapid division. This stimulus, it would seem, takes place through the following delicate adjustment of opposing forces: Within the host cell the sensitive parasite produces as one of its by-products an acid. As this accumulates it stops the growth of the bacteria and destroys a portion of them, without, however, destroying the host cell. The membranes of these dead bacteria, which have now become permeable, allow the diffusion into the host cell of bacterial endotoxines. The host cell now contains, of abnormal bacterial products, (*a*) these escaped endotoxines, (*b*) a certain amount of weak acid (acetic?), (*c*) some ammonia, and (*d*) an excess of carbon dioxide. Under the stimulus of one or more of these poisons the nucleus divides by mitosis. In process of division the nuclear membrane disappears and the contents of the nucleus flows out into the cell. The dormant bacteria under the stimulus of this nuclear substance renew their activities in the daughter cells until again inhibited, whereupon the daughter cells divide. By this rocking balance, in which first the parasite and then the host cell has the advantage, the tumor develops rapidly and independently of the needs of the plant.

The facts underlying this hypothesis are as follows:

(1) *Evidence from pure cultures.*

(*a*) The development of Y-shaped and other involution forms in pure cultures of *Bact. tumefaciens*, subjected to unfavorable conditions.

(*b*) The development of an acid in sugared peptone water cultures, to the action of which acid the involution forms are attributed.

(*c*) Our ability to produce these Y-shaped bodies at will and promptly in young agar and bouillon cultures by addition of small quantities of dilute acetic acid.

(*d*) Proof from agar-poured plates that most or all of the Y-bodies developed in the presence of the acetic acid are dead, i. e., will not grow into colonies when copious sowings are made on agar plates.

(*e*) The observation that those bacteria which are not killed by exposure to the acid are so paralyzed that they come up more slowly on agar-poured plates than do those from untreated cultures.

(*f*) Production of ammonia by this organism in culture-media as one of the results of its assimilation of nitrogen compounds.

(*g*) While *Bact. tumefaciens* does not give off CO_2 in measurable quantities in fermentation tubes in the presence of sugar, it is assumed that such large volumes of the gas are not only not requisite but would be injurious to the mechanism of tumor development, and that all ordinary bacteria, including this one, throw off some CO_2 as a result of their growth.

(2) *Corresponding evidence from the plant.*

(*a*) To obtain *Bact. tumefaciens* from the tumors by means of agar-poured plates unusually heavy sowings must be made.

(*b*) These colonies often come up slowly as if the bacteria from which they have developed were nearly dead and must first recover from some inhibition before they can grow. Once recovered they grow satisfactorily.

(*c*) The existence of good-sized Y-bodies and variously deformed bacteria in the tumor tissue as shown by their diffusion from sliced tissues lying on flamed slides in bacteria-free water.

(*d*) Proof from ordinary sections, stained and unstained, that the bacteria do not occur in the vessels or the intercellular spaces of the tumor. There are no bodies of any sort in these places.

(*e*) Demonstration by impregnation with gold chlorid that the bacteria are not abundant in the tissues, that they occur *inside the cells but outside the nucleus*, and that Y-bodies and variously branched forms are common.

(*f*) The existence of an excess of CO_2 in the tumor cells is inferred from the behavior of the chloroplasts which multiply in the tumor strand and other deep parts of the tumor in large numbers so as to make the tissues decidedly green.

(*g*) A statement by the chemist that the same acid occurs in tumor tissue as in our flask cultures.

Up to this time we have not been able to determine the litmus reaction of the nucleus; neither have we been able to show conclusively that the acid produced in the tumor tissue is identical with that developed from grape sugar in our flask cultures. The uranium salts look much alike, but I am not sure that they are identical. Further studies will be made.

EFFECT OF CHROMATES.

Ever since we have known this neoplasm to be due to bacteria lodged within the cell, it has been a foregone conclusion that, however much the tumor cells might resemble meristematic or embryonal tissues morphologically, their chemical contents certainly must be somewhat different both on account of the by-products thrown off during the growth of the bacteria and by reason of reactions set up by the cells against the intruding organisms. In Bulletin 213, page 173, it was shown that the tumor tissue (sugar beet in that case) contains

an excess of colorless substances oxidizing to dark compounds on exposure to the air. A further evidence of chemical difference is shown on Plate CIV. In figure 1 of this plate the deeply stained pieces represent portions of a daisy tumor soaked in water saturated with potassium bichromate. The unstained pieces are normal parts of the daisy treated in the same way. The brown stain appears slowly in cold solution but within a few minutes in hot ones. Potassium chromate and neutral ammonium chromate have the same action on the galled tissues, staining them a deep brown, whereas the normal meristematic tissues are only feebly stained. Chromic acid and chrome alum did not have this effect. The substance which causes this dark stain may be extracted from the tissues readily by hot alcohol, as shown in figure 2 of Plate CIV. Here the right-hand sections were exposed for a few minutes to the action of a hot saturated solution of potassium chromate. The sections at the left were from the same tumor and were treated in precisely the same way, except that they were first thrown into hot ethyl alcohol and allowed to simmer for a few minutes. That some substance is actually removed from the tissues is shown by the subsequent behavior of the alcohol in presence of these compounds of chromium. This reaction was discovered in making experiments to determine whether the appearance of the gold chloride preparations might not be improved by some preliminary treatment. It was hoped that a way might be found to remove some of the substances causing granular precipitates within the cell and so leave a clearer picture. Naturally we first thought of tannins. What this soluble substance is remains to be determined. Possibly it is a tannin, in which case the brown reaction might perhaps be considered as a phlobaphene reaction due to the acid radicle of the salts used.

NOTES ON TECHNIQUE.

The daisy plants described in the following pages are plants selected from a series inoculated especially for the purpose of studying the movement of the tumor strand and the development of stem structure in the leaves. All were inoculated at about the same time, a foot or more above the earth, and all in the young, rapidly growing stems, except those plants used for checks, i. e., those inoculated on the leaves. All of the inoculations were made by needle pricks without hypodermic injection. At the time of the inoculation the stems were soft and rapidly elongating, and the needle pricks were made in what was then the top of the plant. For inoculating material we used agar subcultures from single poured-plate colonies. All of the inoculated plants contracted the disease where pricked and not elsewhere, except as the result of invasions from the primary tumor.

We obtained a great wealth of pathological material of which only a part could be used for the preparation of this bulletin. The material selected for the sections was fixed in Carnoy's fluid (acetic acid 1, alcohol 3). Suitable pieces were then infiltrated with paraffin, cut on the microtome, usually in series, fastened to clean slides with egg albumen, and stained in various ways, none of which, however, demonstrated any bacteria in the tissues, i. e., not with certainty. We sometimes saw rods in these sections but could not be *sure*.

Many stains were tried. The best stain for differentiating lignified from nonlignified tissues proved to be that recommended by Chamberlain (Methods of Plant Histology, pp. 49 and 68), i. e., a prolonged stain in methyl green followed by a short exposure to acid fuchsin.

The photomicrographs were made on Hammer's double-coated nonhalation orthochromatic plates using monochromatic light (Zettnow's fluid). They were made with Zeiss apochromatic objectives and a No. 4 compensating ocular. All of the photomicrographs except a few at the end were made either with the Zeiss 16-millimeter or 8-millimeter objective. The source of the light was an electric arc. The developer used was rodinol, usually 1 part to 30 parts of water. The exposures were made by the senior writer, but the plates were developed and printed from by Mr. Brewer.

NORMAL ANATOMY OF THE DAISY.

So much will be said about abnormal phenomena in the stems and leaf traces of the daisy that some words of introduction are necessary respecting the microscopic structure of the normal plant, especially for those not familiar with the anatomy of plants.

STEM.

In the center of the stem is a *pith*. This is composed of nearly isodiametric cells which, as growth continues, become compressed in various directions by pressure of neighboring cells so that on cross section they are often hexagonal. This tissue, while remaining alive for a considerable period, remains dormant and functions chiefly as a storage system. At the periphery of the immature stem is a cylinder of coarse-celled, rather loose tissue known as *bark parenchyma*. The young stem is covered and protected from the air by a one-celled layer known as the *epidermis*, between which and the bark parenchyma one or more layers of cells with thickened angles may be present. This is known as *collenchyma* and functions as a strengthening tissue. Finally in this region a tissue develops which is known as the *cork cambium*. As the stem grows this cork cambium proliferates (slowly on the daisy), giving rise to layers of impervious cells (*cork*) which take the place of the thin, temporary epidermis. Between the bark parenchyma and the pith is a complex structure consisting of a series

of elongated *bundles* radiating from the pith in every direction. These are few in number at first and separated by wide areas of looser tissue (Pl. I), but very numerous as the stem grows, and then separated by narrow, compressed plates of nearly isodiametric cells known as *medullary rays.* In the young stem these rays extend from the pith to the bark parenchyma. In older stems new ones arise from time to time and take their origin at more and more remote distances from the pith. Bordering these bundles externally, i. e., on the inner border of the bark parenchyma, is a layer of cells known from its contents as the *starch sheath.* The bundle consists principally of wood and soft bast or *xylem* and *phloem,* in the terminology of the anatomist. The structure in detail of a single bundle in such a stem is as follows: The xylem portion consists, at its older (innermost part), of a few *spiral vessels,* which do not increase in number; beyond these are *pitted vessels* (tracheids) and *wood fibers* (wood parenchyma) which are continually increasing in number as the stem grows. The phloem consists of *sieve tubes* (slime vessels) and *companion cells.* The longer axis of these vessels corresponds to that of the stem. As the stem increases in size *bast fibers* (groups of thick-walled elongated cells) appear in the outer part of the bundle not far from the phloem, i. e., inside the starch sheath. The phloem is separated from the xylem by the *cambium* which also separates the xylem part of the medullary ray from its phloem part. The cambium is the most actively growing part of the stem, giving rise to new bundles and increasing the size of the old ones, xylem tissues being laid down on the inner side of it and phloem tissues on the outer.

Plate I shows a cross section of a healthy portion of Daisy No. 1, branch II, from epidermis to pith. Beginning at the top, the tissues occur in order as follows: *E,* epidermis; *cc,* subepidermal layer; *cp,* cortical parenchyma with chloroplasts; *st,* starch sheath; *bf,* group of bast fibers; *p,* phloem; *c,* cambium; *t, sp,* xylem wedges (tracheids and spirals), which are heavily stained; *mr,* medullary ray; and *pt,* pith. As the stem increases in diameter the wood wedges become numerous and compacted into a thick cylinder, and then the epidermis is slowly replaced by bark. Section stained with methyl green and acid fuchsin.

Plate II shows in cross section a part of branch III, Daisy No. 1. Here the ring of wood is somewhat thicker, and the bast fibers are also more abundant. For appearance in cross section of the older stems containing a much greater quantity of wood, see Plates XXI, XXV, LXIII, LXVII, and LXXVIII.

When leaves or branches are given off from such a stem they include parts from all the various elements of the bundle as mentioned below.

LEAF TRACES.

Those vascular portions of the stem which pass out into leaves are known as *leaf traces*. In the daisy petiole there is a central leaf trace and several side ones. Farther out (on the lamina of the leaf) these leaf traces branch repeatedly, forming a supporting and conducting network, the so-called *veins* of the leaf. The appearance of a normal leaf trace in cross section is shown on Plate III. Beginning at the top of the plate (under surface of the petiole), the tissues occur in order as follows: *par*, loose parenchyma or ground tissue of the petiole; *st*, starch sheath, entirely surrounding the bundle; *scl*, thin-walled elongated supporting cells; *p*, pholem; *c*, cambium; *t*, tracheids of the xylem; *m*, medullary ray; *sp*, spirals of the xylem; *scl*, thin-walled elongated supporting cells; and *par*, surrounding loose-celled parenchyma of the petiole. On Plate IV may be seen the appearance of a similar leaf trace in longitudinal section, the top of this plate corresponding to the top of the preceding. Below the large spiral vessels are smaller spiral vessels and delicate ring vessels. It is through this region that the tumor strand passes. Plate V shows the appearance of a longitudinal section through a petiole between two leaf traces, i. e., through the ground tissue of the leaf from epidermis to epidermis. Portions of this tissue are often surrounded by and incorporated into the growing tumor.

The function of the xylem part of the bundle, aside from support, appears to be storage and movement of water; that of the phloem, storage and movement of elaborated nitrogenous substances. The pith and medullary rays are often used as starch receptacles. The bark parenchyma contains numerous chloroplasts whose function it is to convert water and carbon dioxide into sugar and starch. The pith, young wood fibers, and medullary rays also contain chloroplasts but in lesser numbers.

In considering the abnormal phenomena now to be described these plates illustrating the normal anatomy will be of prime importance.

BEHAVIOR AND HISTOLOGY OF SELECTED PLANTS SPECIALLY INOCULATED FOR THESE TESTS.

DAISY NO. I.

Whole Plant.

(Plate VI.)

This plant was inoculated January 13, 1911, and photographed March 10, 1911. Primary tumors developed at *x*, where punctured. Secondary tumors afterwards appeared on leaves *A*, *B*, *C*, and *D*, as shown. After photographing, agar poured plates were made from the interior of each one of the branches (I, II, III) to determine

whether the inoculated organism could be isolated therefrom. Plates were also poured from the secondary tumors on *A*, *B*, and *C*. Finally, portions (marked *F*) of the three branches and of leaf *C* were fixed, embedded, sectioned, and stained for demonstration of the tumor strand, if such existed.

The poured plates were as follows:

A (not burst through), 8 plates (Brown).
B (burst through), 5 plates (Hedges).
C (burst through), 7 plates (Brown).
I, 8 plates (Brown).
II, 6 plates (Hedges).
III, 7 plates (Brown).

Total, 41 plates. All negative or doubtful. One of the colonies which came up on the twelfth day from stem I looked hopeful and was transferred, but there is no record of inoculations.

The histology of the parts is shown in the following plates.

Branch III.

(Plate VII.)

Cross section of branch III, slide 575 C 1, in the lower part. This section shows the junction of wood and pith with a small tumor strand in the center of the picture. This strand passes from the primary tumor into petiole *C*. Section torn in mounting. For a normal part of the same section, see Plate II. For longitudinal sections of this strand farther away from the primary tumor, i. e., in the petiole, see Plates XVII to XX.

Petiole C.

(Plates VIII and IX.)

Cross section of petiole *C* (Pl. VIII), at one end of the lower fixed portion (slide 643 A 18), showing the enlarged and modified central leaf trace. The traces on either side of this one are normal. The parenchyma between the leaf traces is also normal, but is somewhat shrunken by the fixing agent.

In Plate IX the upper part of the middle leaf trace, slide 643 A 19, is enlarged to show the tumor strand. For a similar section from the other end of the fixed part of this petiole, see Plates X and XI, which should be compared with Plate III.

Petiole C.

(Plates X and XI.)

Plate X.—Cross section of central leaf trace at other end of the fixed portion of petiole *C*, slide 643 B 6, showing abnormal wood wedges. The original leaf trace is at the lower and left part, some-

what enlarged and distorted, but otherwise nearly unchanged. The upper portion of this leaf trace enlarged to show the tumor strand may be seen in Plate XI, made from another slide in the same series (643 B 12).

BRANCH I.

(Plates XII, XIII, and XIV.)

The varying appearance of the tumor strand at various levels in the same series of sections is well illustrated in Plates XII to XIV, made from branch I under petiole *A*. (See Pl. VI.) Here the tumor strand (block 575 A, slides 8, 10, and 11) is in the inner wood next to the pith. In Plate XII it consists exclusively of small cells. In Plates XIII and XIV there are also in it large soft cells with very conspicuous nuclei. In Plate XIV, in the lower part of the strand, are tracheids developed from cells of the tumor strand and twisted at right angles to their normal direction. This is a common occurrence.

BRANCH II.

(Plate XV.)

Cross section of branch II, slide 575 B 10, just under petiole *D*, showing a somewhat irregular tumor strand in the xylem part of the bundle. In the lower part of it, near the pith, are twisted tracheids developed from the tumor strand. Above is infiltration.

PETIOLE C.

(Plate XVI.)

Tumor tissue from petiole *C*, slide 643 C 39, showing many cells with 2 nuclei. It is all rapidly proliferating parenchyma, but the cells are growing in different directions.

PETIOLE C.

(Plates XVII, XVIII, and XIX.)

Plates XVII to XIX are sections of the upper portion of the fixed part of petiole *C*, i. e., they are the same as Plates IX and XI, but cut longitudinally to show the extension of the tumor strand. The plates were made from block 643 *c*, slides Nos. 23, 21, 19, and 17. The top of the plate represents the proximal part of the petiole. The strand, which comes from the stem, has developed the small unruptured tumor *M* (Pl. VI), and thence passed into the part here shown. The small, rapidly proliferating neoplasm shown at the bottom of Plate XVIII is not yet large enough to be indicated by any surface swelling. In XVII and XVIII, *x* corresponds to *x*. The distal part of the strand in greater detail is shown on Plate XX.

PETIOLE C.

(Plate XX.)

Plate XX shows a longitudinal section of petiole *C*, one field beyond Plate XIX, i. e., its top joins on to the bottom of Plate XIX. In the center of the plate is the tumor strand, to the left are spiral vessels, to the right is a portion of the tissue marked "scl" in Plate III, with which, and Plate IV, this should be compared.

DAISY NO. II.

CROSS SECTION OF STEM.

(Plate XXI.)

One of the plants inoculated January 13, 1911, and fixed March 23, 1911. This plate gives a cross-section of the stem, slide 641 A 13, between the primary tumor and a secondary one and shows a well-developed tumor strand on the lower right-hand side at the junction of wood and pith. The wood is enlarged a little near the strand, but otherwise it is normal. The bark is also normal. The pith cells are flattened next the tumor strand by pressure, but farther away are normal. For a detail see next plate.

TUMOR STRAND IN STEM.

(Plate XXII.)

Plate XXII, from slide 641 A 13, gives an enlargement of the tumor strand shown on Plate XXI. The black dots are nuclei. The figure shows a vague demarcation at the top where the tumor strand shades into the modified wood, and a sharp one below where the pith cells are flattened by pressure. At *sp.* are several rows of crushed spiral vessels; at *X*, *Y*, *Z*, are displaced spiral vessels. This section was stained with methyl green and acid fuchsin.

DAISY NO. V.

WHOLE PLANT.

(Plate XXIII.)

This plant was inoculated January 13, 1911, and photographed March 27, 1911, three-fourths natural size. Primary tumor at *X* where the needle pricks were made. Secondary tumors on leaves *A*, *B*, and *C*. Both *A* and *C* have split open. Several small roots were projecting from the base of the primary tumor on this side, but are hidden by the apex of leaf *B*. For the back view of this plant, see Plate XXIV.

WHOLE PLANT, BACK VIEW.

(Plate XXIV.)

Plate XXIV gives the opposite side of the plant to that shown on the preceding plate. *A* and *C* correspond. *B*, which has been shortened to a stub, is invisible. At *D* are secondary tumors in the base of a leaf, the remainder of which has shriveled under the influence of the disease. Four leaves on this side between *C* and *A* were free from visible growths. They were removed before photographing for clearness sake. Roots stimulated into development by the presence of the tumor are present at *RR*. The stem between *E* and *F* was removed; photographed end on, at *F* (see lower left part of the plate); and then split longitudinally, along the median line (see lower right part of plate). In the middle of the vertical section is an expansion of the green tumor strand which later on might have burst through the wood and bark and appeared as a surface tumor. Secondary stem tumors of this sort are now present in the hot houses. The cross section shows enlargement of the wood on one side and a distinct green tumor strand between wood and pith. For the minute anatomy of the stem at this level, see Plate XXV.

CROSS SECTION OF STEM.

(Plate XXV.)

Cross-section of stem, slide 594–8, at the level of *F* in Plate XXIV, showing the large tumor strand with thickened wood cylinder on that side only and absence of thickening in the bark. Under a hand lens the duplication of medullary ray cells near the strand may be seen in the photograph but is lost in the half tone. The pith is normal except in the vicinity of the tumor strand where it is flattened.

Section stained with Ehrlich's acid hematoxylin. The strand is redder than the other tissues.

For details from the tumor strand see Plates XXVI and XXVII.

A DETAIL FROM THE TUMOR STRAND.

(Plate XXVI.)

Plate XXVI, from slide 594–8, gives more highly magnified the upper portion of the tumor strand shown in Plate XXV. At the left are the cells of the tumor strand; at the bottom, top, and right side is the modified wood. The demarcation between the modified xylem and the tumor strand is somewhat vague. The black dots are deep-staining nuclei.

DETAIL FROM THE TUMOR STRAND.

(Plate XXVII.)

Plate XXVII, from slide 594–8, gives the lower part of the tumor strand shown on Plate XXV. The cells of the tumor strand are at the right. The pith is at the left and its cells have been flattened by

the pressure. The demarcation between the tumor strand and the pith is sharp.

DAISY NO. VII.

Whole Plant.

(Plate XXVIII.)

This plant was inoculated on January 13, 1911, and photographed March 31, 1911, about two-thirds natural size. The bacterial culture was introduced at *X X*, where the primary tumor soon developed. Secondary tumors developed on leaves *A*, *B*, *C*, *D*, and *E*, and also in the interior of branches I and II. Material at *F* was fixed for microtome sections from stem I and from leaves *A* and *D*. Petri-dish plates were poured from the interior of *A*, *B*, *C*, and D. Pieces from the interior of I and II were also transferred to tubes of bouillon and incubated.

The plant was 8 months old and 24 inches high. It divided into two equal branches, both of which were inoculated. One branch only is shown in the photograph (for notes on the other one see below). The inoculation was made 11 inches from the ground. The actual distances at the time the photograph was made were as follows: Origin of leaf *A* to primary tumor, 6 cm.; length of infected portion of leaf *A*, 9 cm.; origin of *A* to base of *B*, 5.5 cm.; base of *B* to its tumor, 4 cm.; total distance from primary tumor to tumor on *B*, 15.5 cm.; time, 77 days; top of *C* to origin of branch bearing it, 5 cm.; origin of *D* to junction of II with main stem, 4 cm.; length of secondary tumor on *D*, 6 cm. At *Y Y* the petioles in cross section are circular and resemble stems, i. e., they have a narrow green pseudopith, a wide ring of wood, and beyond this a cambium and phloem. All that distinguishes *D* as a petiole are the unchanged petiole wings. For the appearance of microtome sections from these petioles see the following plates.

Both above and below the swelling on I there is a thickening of the wood on one side, and in the inner part of this next to the pith is a green strand of tumor tissue about 1 mm. in diameter.

The following are notes on the inoculated branch which was not photographed:

The primary tumor is now 2 by 1.5 inches in diameter. The main axis has been dwarfed by the growth of the primary tumor to a tiny shoot 3 inches high. This is 2 to 3 mm. in diameter, except at the base, which is much swollen by an internal tumor not yet ruptured to the surface (this part is 1 cm. in diameter). Four centimeters above the base a leaf arises which bears secondary tumors in the midrib for a distance of 5 cm. Under the primary tumor, coming out of the wood and bark is a cluster of nine small roots. On the back side, in the same relative position, are eight other small roots.

The primary tumor has also grown into the base of three other shoots as follows: (1) Right-hand shoot, arising from the middle of primary tumor, where all but the base of the shoot has been destroyed, the latter being occupied by a living tumor 3 cm. long and 2 cm. broad. (2) Left-hand shoot, arising near base of primary tumor, bears a ruptured tumor for a distance of 4 cm. and thence appears sound externally for a distance of 10 cm., where it gives off a leaf that at a distance of 7 and 8 cm., respectively, from its junction with the stem, shows on the midrib two unruptured secondary tumors, the remotest one being, therefore, 22 cm. from the primary tumor and 17 cm. from any surface indications of disease. (3) In the middle back part of the primary tumor a stem arises which shows no surface tumors, but is swollen and bears two leaves with secondary tumors, diameter of stem at base being 5 mm., but 2 cm. up (in swollen place) it is 8 mm. in diameter. The first diseased leaf arises at a distance of 5 cm. up and shows ruptured tumors for a distance of 6 cm. on the midrib. Two centimeters farther up the second leaf arises and this bears secondary tumors on the midrib for a distance of 2 cm.

The tumor stimulus has extended down the main stem, but much less conspicuously. It is visible for a distance of 8 cm. below the main tumor, as shown (1) by a root pushing out 3.5 cm. below, and (2) by a small secondary tumor in a leaf scar 8 cm. down. Here the stem is 1.5 cm. in diameter and quite woody. In a hasty examination I could find no tumor strand in the interior of this hard stem.

The cultures were negative or doubtful, as follows: Petiole *A*, no gall colonies; petiole *B*, no gall colonies; petiole *C*, one gall colony, daisy inoculation therefrom negative; petiole *D*, no gall colonies; stem I, no gall colonies on plates poured from three tubes after clouding; stem II, after six days very suggestive stringy filaments in both tubes. Plates from one gave only pink colonies, plates from the other gave colonies which for a time very much resembled the daisy organism, but we finally rejected them as intruders. Two of the four tubes of II inoculated with large pieces of the stem never clouded (17 days), and also one of the four of stem I.

Petiole A.

(Plate XXIX.)

Plate XXIX, from slide 592 B 17, gives the base of petiole *A* in cross section, showing conversion of the central leaf trace and two side traces into secondary tumors having stem structure. A tumor strand occupies the center of each modified leaf trace. The only unchanged parts of the petiole are the periphery and the marginal wings. The primary xylem of the leaf trace occupies about one-fourth of the present wood cylinder, i. e., that on the right side.

For a detail from the center of the middle tumor see next plate. Section stained with methyl green and acid fuchsin.

PETIOLE A.

(Plate XXX.)

Plate XXX, from slide 592 B 17, gives the center of the middle leaf trace from Plate XXIX enlarged to show the character of what may be called the pseudopith, i. e., the rapidly proliferating tumor strand, in the center of the pseudostem. Woody tissues at top and bottom. In the lower right corner an abnormal medullary ray.

PETIOLE D.

(Plate XXXI.)

Cross section of petiole *D*, slide 592 A 15, showing conversion of the petiole into a secondary tumor which has not yet ruptured to the surface. Stem structure plainly visible. Several infected leaf traces have fused. The central tumor strand is conspicuous. The margins of the petiole are not involved; but for these no one would suspect this to be a petiole. The actual diameter of the mounted section is 8 mm. The primary xylem of the leaf trace appears to be on the lower right hand side. Section stained with Ehrlich's acid hematoxylin.

DAISY XI.

WHOLE PLANT.

(Plate XXXII.)

This plant was inoculated January 13, 1911, and photographed April 5, 1911, about three-fourths natural size.

The primary tumor, *X*, *X*, induced by needle pricks, surrounds the stem except as here shown. Secondary tumors developed after some weeks on *A*, *B*, *C*, and *D*. Leaf *D* has shriveled to a stub. The growth in *A* has ruptured to the surface but not in *B* except near the apex. The terminal part of *B* was removed earlier for study. On dissection a green strand of soft tumor tissue was traced from the primary tumor into both *A* and *B*. The petiole *B* in the vicinity of *M* was found to have stem structure in the middle leaf trace and was fixed for sections (Pl. XXXIV). In the main stem at *P*, i. e., just under the attachment of leaf *C*, a green strand of tumor tissue was visible under the hand lens, but was inconspicuous. For the appearance of this strand when more highly magnified see Plate XXXIII.

Cross Section of Stem.

(Plate XXXIII.)

Plate XXXIII, from slide 591 AA 3, gives a cross section of the stem at the lower line of *P*, i. e., just under origin of the infected leaf *C*. It shows an abnormal soft tissue strand in the wood somewhat farther away from the pith than customary. The vessels of the single row below this strand are spirals. Pith cells at the bottom. Stained with methyl green and acid fuchsin.

Petiole B.

(Plate XXXIV.)

Plate XXXIV, from slide 591 B 8, gives a cross section of base of petiole *B* showing stem structure in the central leaf trace. In the center of this tumor is the tumor strand; to the right is the original xylem of the leaf trace more broken up and disorganized than in Plate LII, i. e., there appears to have been an invasion of soft cells from the tumor strand or a stimulus to increased development of the medullary rays from proximity to that strand. In other directions may be seen the abnormal wood wedges separated by wide areas of nonlignified tissues—medullary rays, etc. Beyond is cambium and beyond that phloem. At the extreme right beyond (outside) the phloem are a few thin-walled pitted vessels in what appears to be a small modified misplaced leaf trace, or portion of such a trace.

The small black specks in the central strand are normal and crushed spiral vessels wedged off from the base of the primary xylem of the leaf trace by the growth of the tumor tissue. These appear to be a portion of the original spirals of the leaf trace. All the *new* vessels forming the abnormal wood wedges are tracheids, i. e., short pitted vessels. These are stained blue in the section and are dark in the photograph.

Petiole B.

(Plate XXXV.)

Plate XXXV, from slide 591 B 6, gives the middle portion of the modified middle leaf trace of petiole *B* at about the same level as Plate XXXIV, enlarged to show the character of the tumor strand which contains both pitted vessels and spiral vessels. The primary wood wedges of the leaf trace are in the upper right hand corner. Projecting into the tumor-strand from this part crushed spiral vessels may be seen. Section stained by Gram-eosin.

Petiole B.

(Plate XXXVI.)

Plate XXXVI, from slide 591 B 2, gives a cross section of the middle portion of the infected leaf trace of petiole *B* in vicinity of *M*, showing two of the three whorls of small cells surrounded by tracheids.

These are on that margin of the tumor strand remotest from the primary xylem of the leaf trace. They are not visible on Plate XXXV. Section stained with methyl green and acid fuchsin.

In the middle of the figure on the right side are tracheids twisted at right angles to their normal direction.

DAISY XII.

Whole Plant.

(Plate XXXVII.)

Plant inoculated by needle pricks on January 13, 1911, and photographed April 5, 1911, nearly natural size. Primary tumor, *X*, *X*, mostly on the reverse side of the plant where pricked. Secondary tumors on petiole *A*, which has ruptured, and on *B*, which has not yet broken open. Behind *A* was a third leaf occupied by secondary tumors and now shriveled to a stub. In cross section at *M* the petiole *A* shows stem structure, i. e., a green pseudopith, greenish wood cylinder (complete), cambium ring, and phloem. In the lower part of the picture (magnified about 2 diameters) is a cross section of petiole *A* where at *X* and *Y* are shriveled remnants of the wings of the petiole. This may be compared with the earlier stage shown in Plate XXXIV and the still earlier stage on Plate XLVII. Here the petiolar tissue outside of the tumor is entirely destroyed. Although *A* is circular in cross section at *M* and has a diameter of 9 mm., it is attached to the stem by a small, shriveled pedicel, i. e., the remnant of the petiole base. The stem was split along the vertical line and then cut crosswise at *O* to O^3.

At the level of *O*, deep in the stem, was a soft, green strand of tumor tissue nearly circular and about 1 mm. in diameter, proceeding from the primary tumor toward *A*. Under the microscope this appeared to be totally unlike either wood or pith. It was on the tumor side of the stem in the wood, but near the junction of wood and pith. This tumor strand was also visible to the naked eye or with the hand lens at O^2 and O^3, but not at O^1. However, when fixed, embedded, sectioned, stained, and examined under the compound microscope, it was visible here also (see Pl. XXXVIII). For appearance of petiole *B* in cross section at various levels, see Plate XLII and the following ones.

Cross Section of Stem.

(Plate XXXVIII.)

Plate XXXVIII, from slide 590 $C^1$1, gives a cross section of the stem at O^1 (see Pl. XXXVII), showing a tumor strand at the inner border of the wood next the pith. Some of the pith cells at the bottom of the picture are shriveled, owing to the fact that the stem

segment was cut diagonally and these cells border on the extreme edge of the cut. Above this strand, in a vertical line, is a row of spiral vessels (walls stained blue). On a right line with these in the lower part of the strand is a lignified vessel of another type (tracheid), the blue wall being represented by a wide black border (it is the immature cell containing an elliptical nucleus). The walls of the other bordering cells and of the strand cells in the section are stained red. Other sections in this series also show tumor-strand cells in process of conversion into pitted vessels.

Section stained with methyl green (for the xylem) and acid fuchsin (for the protoplasm and cellulose walls).

PETIOLE A.

(Plates XXXIX and XL.)

Frequently inside a tumor several proliferation centers of soft, small cells may be seen surrounded by whorls of tracheids, as shown in Plates XXXIX and XL, made from different areas in the same slide (590 A 7). Cut at right angles to sections here shown, they appear as strands, and which often pass out toward the surface of the growth. There are two of these whorls in petiole *A* at the level of this section. They are near the center on that side of the tumor strand (Pl. XLI) remotest from the original xylem wedges of the leaf trace. The top of each picture is toward the periphery of the tumor, and they join onto each other (*X* corresponding to *X*) and also join on at their bottom to the top of Plate XLI, which shows the tumor strand.

PETIOLE A.

(Plate XLI.)

Plate XLI, from slide 590 A 2, gives a cross section of the central part of the secondary tumor in petiole *A* (Pls. XXXIX and XL), showing rapidly proliferating large cells of the tumor strand, containing spirals (the thick-walled cells). At the bottom and left the innermost elements of the wood wedges are visible. The bulk of the xylem is below, the wood cylinder being open and imperfect above.

PETIOLE B.

(Plate XLII.)

Plate XLII, from slide 590 B 13, shows a cross section of petiole *B* in the vicinity of fig. 13 (Pl. XXXVII). Above, below, and at the left are the unchanged petiolar tissues. In the center and at the left is the enlarged central leaf trace, preserving nearly its normal form (compact tissue at left of center). At the base of this, in the

center of the section, is the tumor strand, radiating irregularly from which may be seen additional abnormal wood wedges. Toward the right may be seen masses of small-celled tumor tissue inclosing numerous unmodified cells of the loose parenchyma of the petiole. There are also tracheids near *X* and *X*. The leaf trace next under this one is also distorted. For a detail of the tumor strand see next plate.

PETIOLE B.

(Plate XLIII.)

Plate XLIII, from slide 590 B 10, gives the central part of a section from nearly the same level as the preceding (Pl. XLII), showing (in the center) the large soft cells of the tumor strand at the base of the primitive wood wedges of the leaf trace. The larger cells below are inclusions.

PETIOLE B.

(Plate XLIV.)

Plate XLIV, from slide 590 B 16, shows a cross section of the secondary tumor in petiole *B* at some distance from fig. 13 (Pl. XXXVII), Here the tumor strand is larger and the abnormal wood wedges are more prominent than on Plate XLII. For a detail of the tumor strand see next plate.

PETIOLE B.

(Plate XLV.)

Same as preceding plate (i. e., from slide 590 B 16), but the central part more highly magnified to show the character of the central tumor strand. The bottom of this picture corresponds to the left of the preceding. In the upper part of the strand is a whorl similar to those shown on Plates XXXIX and XL. At the right of it is an unmodified medullary ray consisting of one row of small cells and below this a modified one consisting of several rows of large soft cells. The tumor strand contains both large and small celled parenchyma.

PETIOLE B.

(Plate XLVI.)

Plate XLVI, from slide 590 B 19, shows a cross section of the middle leaf trace of petiole *B* beyond any visible swelling, i. e., somewhere near figure 19, Plate XXXVII. Here the secondary tumor is reduced to a comparatively small mass of tissue, i. e., to the tumor strand at the base of the xylem wedges, and a small mass of tissue below it. For appearance at the lower end of this petiole see the next two plates.

PETIOLE B.

(Plate XLVII.)

Plate XLVII, from slide 590 B 24, shows a cross section of petiole *B* near its base, i. e., at about the level of figure 24, Plate XXXVII. The external shape of the petiole is about normal, the upper surface of it being at the right. Only the central leaf trace is here diseased, for details of which see next plate. Section stained by Gram-eosin.

PETIOLE B.

(Plate XLVIII.)

Plate XLVIII, from slide 590 B 24, gives enlarged the middle leaf trace from the preceding plate, showing the beginning of the pseudostem (secondary tumor). In the center is a group of soft cells forming the tumor strand. This consists of mixed tissue, many of the cells being isodiametric or only slightly longer than broad, while others are more or less elongated. Above is the leaf trace slightly modified, i. e., showing in its lower part infiltration of soft cells from the tumor strand, wedging apart the spiral vessels. Below are abnormal wood wedges and connective tissue. The vessels of this part are tracheids. On the outer edge at *S* are groups of sieve tubes. To determine the origin of these abnormal wood wedges the remainder of the petiole was sectioned longitudinally, i. e., downward toward the stem. (See Pl. XLIX.)

PETIOLE B.

(Plate XLIX.)

Longitudinal section, slide 590 B_2 21, of abnormal part of leaf trace shown in Plate XLVIII. Beginning at the bottom we have: *sp*, normal spirals of the leaf trace; *tstr*, tumor strand; *scl*, normal supporting cells of the leaf trace; *tr*, abnormal tracheids developed from the tumor strand; *tp*, tumor parenchyma; *inc*, included cell, i. e., one of the large cells of the loose parenchyma of the petiole.

PETIOLE B.

(Plate L.)

Cross section of petiole B, slide 590 B 4, of Plate XXXVII, showing abnormal lignification of the walls of four of the included cells. These are large cells of the loose parenchyma of the petiole, like those shown in Plates XLII and XLIV. They have taken a deep blue stain (lignin), whereas the walls of similar cells below are pink (cellulose). Section stained with methyl green and acid fuchsin. Such abnormal lignification of parenchyma cells is not infrequent.

DAISY NO. XIII.

Whole Plant.

(Plate LI.)

This plant was inoculated on January 11, 1911, and photographed April 6, 1911, seven-eighths natural size. The inoculations were by needle pricks at *X*, *X*. The stem is swollen in the vicinity of the primary tumor. *A* and *B* are leaves bearing secondary tumors. *B* is directly over the right side of the primary tumor and 8 cm. away. The secondary growths on this leaf which have split it open in places extend outward a distance of 10 cm., making a total extension from the primary tumor of 18 cm. in 54 days. The stem between *A* and *B* is normal externally, except that at the level of figure 2 on a vertical line between the primary tumor and *B* there is a very slight swelling of the stem over the location of the tumor strand.

The internal condition between the right side of the primary tumor and *B* in a straight line is as follows: At 1, slightly thickened wood ring on the right side and a half dozen aggregated tiny spots at the junction of the wood and pith where the tissue is stained greener (best seen in thin section). At 2, the green strand under the swollen wood is more conspicuous and measures 1.5 mm. wide by 0.7 mm. thick (photographed slightly enlarged); see the lower part of this plate. At 3, the wood is not conspicuously enlarged and the strand is not visible under the hand lens. At 4, the strand again shows. Shaved tangentially between 3 and 4, we could not distinguish the tumor strand clearly by its green color.

Petiole B.

(Plate LII.)

Plate LII, from slide 589–3, gives a cross section of petiole *B* near its base, showing a secondary tumor in the central leaf trace, which is greatly enlarged and converted into a pseudostem. In its center is the tumor strand; at its right is the nearly normal xylem of the leaf trace. Radiating from the tumor strand in other directions are the abnormal wood wedges with broad bands of soft cells between them. The tumor is beginning to rupture to the surface on the left side. The cells of the petiole immediately surrounding the tumor are flattened by pressure. Gram-eosin stain. For a detail from the center of the tumor at this level, see next plate.

Longitudinal sections were made through the central leaf trace and sieve tube tissue demonstrated on the periphery of the tumor in nearly all of them.

Petiole B.

(Plate LIII.)

Plate LIII, from slide 589–12, gives the tumor strand and surrounding tissues from petiole *B* at the same level as Plate LII, enlarged to show in detail the character of the tissues. The black dots are nuclei. Sections stained with Ehrlich's acid hematoxylin. The tumor-strand has stained much redder than the surrounding tissues.

DAISY NO. XIV.

Whole Plant.

(Plate LIV.)

This plant was inoculated January 11, 1911, at *X* by needle pricks, and photographed April 6, 1911, three-fourths natural size. Leaves *A*, *B*, *C*, and *D* bear secondary tumors. The distance from the base of *A* at the top of the primary tumor to the origin of *D* is 6.5 cm. The length of the secondary tumor on *C* is 10 cm., that of the ruptured part, 6 cm. The structure of *C* in cross section is that of a stem with a complete wood cylinder, on one side of which is unchanged petiole structure (see Pl. LV). The tumor strand is sometimes in a central leaf trace and sometimes as at *B* and *D* in side traces.

At the level of fig. 2 there is a thickening of the wood and a green stain under both *B* and *C*. At 3 this is less conspicuous. At both of these levels, as seen under the microscope, the area of disturbance is not large, and both it and the green stain were confined to the inner wood next the pith.

Poured plates were made from the interior of *C* above the lower fixed portion and these yielded typical gall colonies in small numbers. On April 19, with agar subcultures from one of these colonies, five young daisy plants were inoculated. At the end of 20 days galls were forming on them at all of the inoculated places. Cultures from this colony into peptonized beef bouillon also gave the typical strings or filaments.

Petiole C.

(Plate LV.)

Plate LV, from slide 593 A 13, gives a cross section of petiole *C*, showing three of the leaf traces involved in a secondary tumor which has ruptured to the surface at the bottom and right and which shows a distinct stem structure, the wood wedges being separated in places by conspicuous plates of modified soft medullary rays (for a detail of these see Pl. LVI). Unmodified petiole tissue occurs at the left and at the top. Above the tumor strand in the lowest leaf trace is a wedge of tracheids in the outer portion of which the longer axis of the

vessels extends parallel to the surface of the cut and tangential to the stem. Slide stained with methyl green and acid fuchsin.

PETIOLE C.

(Plate LVI.)

Plate LVI, from slide 593 A 22, shows a cross section of a small part of the secondary tumor in petiole *C*. At the left is a wood wedge with tracheids of normal arrangement, at the right is a wood wedge with tracheids twisted at right angles to the normal direction. Between these two is an abnormally broad medullary ray composed of soft rapidly proliferating cells, several of which contain two nuclei. In portions of such plates the ray cells (?) are twisted so that their long axis is parallel to the surface of such a cut as that here shown, i. e., like the right wood wedge. Section stained with methyl green and acid fuchsin.

PETIOLE C.

(PLATE LVII.)

Plate LVII, from slide 593 A 22, shows in cross section a woody part of the secondary tumor in petiole *C*, not far from the tumor strand. The central loosely arranged soft cells are possibly modified ray cells. They take the fuchsin stain less deeply and are much larger than the cells of the tumor strand (see next plate) which is below and at the right just outside the limits of this plate.

PETIOLE C.

(PLATE LVIII.)

Plate LVIII, from slide 593 A 22, shows a cross section of the middle part of the secondary tumor in petiole *C*. In the center is the tumor strand. This consists of small rapidly dividing cells which have stained heavily with the acid fuchsin. Surrounding this is a small portion of the pseudostem (for orientation see center of Plate LV). At *A* and *B* are modified medullary rays; at *C* and *D* are woody tissues twisted in abnormal directions. In this plate and the preceding one, *D* corresponds to *D* and the magnification is the same.

PETIOLE B.

(PLATE LIX.)

Plate LIX, from slide 593 B 2, gives a cross section of petiole *B*, showing a secondary tumor with stem structure in *X*, a lateral leaf trace. The leaf trace next below it is also slightly involved, as may be seen more clearly from Plate LX. The rest of the petiole appears to be normal.

Petiole B.

(Plate LX.)

Plate LX, from slide 593 B 6, gives in cross section a portion of petiole *B*, showing the leaf trace next under *X* of Plate LIX. Here the abnormal phenomena is restricted to distortion of the bundle with the separation of a few tracheids from their fellows, the twisting of others at right angles to their normal directions, and the appearance below of wedges of soft tissues.

DAISY XVI.

Whole Plant.

(Plate LXI.)

This plant was inoculated by needle pricks on January 13, 1911, and photographed April 10, 1911, nearly natural size. Primary tumor at *X* where punctured. Secondary tumors at *A*, *B*, *C*, *D*, *E*, and *F*. At *B* and *D* are stubs of leaves removed earlier for study and from the cut surface of which tumors have developed. At *C*, *E*, and *F* are remnants of leaves destroyed by the tumor. On section the base of *A* was found to have a stem structure.

In the stem, under *A* and leading up to it, was a green strand of soft tumor tissue. This was situated at the junction of wood and pith, but mostly in the wood. It continued beyond *A*, i. e., at fig. 2 on the vertical line, and still more conspicuously at 3, but less so at 4; it was visible as a small mass of soft, deep green parenchyma. There was a similar strand under *C*. On cross section this strand pushed out 0.5 mm. as though it had been under great pressure (see Pl. LXII). It contained so many chloroplasts that it was very green in comparison with the color of the wood and pith.

Stem and Leaves.

(Plate LXII.)

Figures *A* and *B* are enlargements of the stem between tumors: *A* gives a cross section of stem at the level of fig. 3 (Pl. LXI) under leaves *E* and *F*, showing a nearly circular strand of soft green tumor tissue with the woody cylinder conspicuously thickened on that side. The difference in color enabled us to get a photograph showing marked contrasts. ×2.

B is the same as *A*, but somewhat further enlarged and photographed obliquely to show more clearly the pushing out of the tumor strand on removal of the pressure.

These are photographs of the specimen exhibited at the meeting of the American Association for Cancer Research, Buffalo, N. Y., April 13, 1911.

Figures *C* and *E* are enlargements of the reverse side of petioles *C* and *E* (Pl. LXI), showing how these deep-seated secondary growths have split open the petiolar tissues and come to the surface. In cross section these growths appear as a flattened imperfect woody cylinder. Photographed April 10, 1911. ×3.

ENLARGED CROSS SECTION OF STEM.

(Plate LXIII.)

Plate LXIII, from slide 586 A 15, gives a cross section of the stem at the level of fig. 2 (Pl. LXI), showing the tumor strand and a conspicuous enlargement of three-fourths of the woody cylinder. The strand is the dark area on the left side of the pith. The section was torn a little in mounting.

A PORTION OF THE TUMOR STRAND.

(Plate LXIV.)

Plate LXIV, from slide 586 A 30, gives an outer portion of the tumor strand in cross section. It is at the same level as Plate LXV, but from another part of the strand. In the upper and left-hand part are distorted tracheids. The spiral vessels except the few mentioned below are at the right beyond this field.

A PORTION OF THE TUMOR STRAND.

(Plate LXV.)

Plate LXV, from slide 586 A 30, gives the inner margin of the tumor strand in the stem, showing the stroma (pitted vessels) originating in the tumor tissue. Below this field is the pith; at *S*, *S*, are spiral vessels of the normal stem widely separated from their fellows by the intrusion of the tumor strand. The remainder of the spiral vessels lie in the direction of the arrow the width of another whole field away.

DAISY NO. XVII.

WHOLE PLANT.

(Plate LXVI.)

This plant was inoculated January 13, 1911, by needle pricks on the stem and photographed April 10, 1911, natural size. Primary tumor at *X*, *X*, where punctured, portions of it beginning to decay. At *A*, *B*, *C*, and *D*, are leaves showing secondary tumors. *A* and *C* are affected only at the base; *B* is diseased through a length of 10 cm.; *D*, which was removed earlier for study of its tumors, has a tumor projecting from the cut surface, i. e., an outgrowth of the diseased leaf trace. The stem at *F* was fixed for sections. The tumor in *C* was also fixed. It showed stem structure at its base.

Petiole *B* showed an imperfect woody ring. The petiole was split open below at *Y* with tumor tissue protruding the same as shown in Plate LXII from plant XVI. Under *B* in the stem at *E* there was a wedge-shaped thickening of the woody ring and a distinct green tumor strand in the inner wood. Plate LXVII made from the stem at the level of *F* shows a smaller tumor strand passing toward *D*.

Cross Section of Stem.

(Plate LXVII.)

Plate LXVII, from slide 639 A 19, gives a cross section of the stem at one end of *F* (see preceding plate), showing a small tumor strand passing to leaf *D*. This is in the inner wood jutting into the pith in that portion of the woody ring (lower part at *X*) which is thickened a little. This thickening was more conspicuous a half centimeter farther down, i. e., under *B*. For a detail of this strand see Plate LXVIII. The diameter of this section is 7.5 mm. The dark places in the bark are normal.

Tumor Strand in Stem.

(Plate LXVIII.)

Plate LXVIII, from slide 639 A 20, gives a detail from the same level as Plate LXVII, showing (in the center) the tumor strand with tracheids developing in it. This is the more interesting because the vessels immediately above the strand (all shown in this photograph) are spirals. Pith below. Both this section and the preceding were stained with methyl green and acid fuchsin.

Tumor Strand in Stem.

(Plate LXIX.)

The same as the preceding but cut from the other end of the stem at the level of *F*, slide 639 AA 21. Pith below, wood above, tumor strand in the center. Section torn a little at the bottom.

The vessels of the wood immediately above this strand are spirals. Just below the strand are four spirals wedged away from their fellows by the growth of the strand. There is also one crushed spiral nearer the strand and two or three displaced ones at the left. No tracheids are visible in it or near it.

Petiole C, Showing Tumor Strand.

(Plate LXX.)

Base of petiole *C*, slide 639 B 10, showing the tumor strand in longitudinal section. Vessels and other tissue lie to either side.

TUMOR TISSUE FROM PETIOLE C.

(Plate LXXI.)

Tumor tissue from the base of petiole *C*, slide 639 B 5, showing cells with 2 and 3 nuclei. On the right are tracheids mingled with tumor cells. The elongated cells at the left are tumor cells cut parallel to their longer axis. There are probably two types of tumor cells in this section. Stained with methyl green and acid fuchsin.

INFILTRATION OF TUMOR TISSUE IN PETIOLE C.

(Plate LXXII.)

Margin of a secondary unruptured tumor in petiole *C*, slide 639 B 9, showing infiltration of the tumor cells into the coarse-celled tissue on the periphery of the petiole. The tumor is at the left, the parenchyma of the petiole at the right. In the upper part, at the extreme right, the epidermis *E* is visible. Outer portion of the petiole curved by the internal pressure.

DAISY No. XVIII.

WHOLE PLANT.

(Plate LXXIII.)

This plant was inoculated January 13, 1911, by needle pricks at *X*, *X*, where the primary tumor developed, and photographed April 10, 1911, nearly natural size. Secondary tumors developed on leaves *A* and *B*. The tumor on *A* is in a marginal leaf trace. The tumor strand of *B* is in the central leaf trace and six tumors developing from it have reached the surface, the remotest one being 9.5 cm. from the stem. Both branches of this leaf (*X* and *Y*) are diseased. The insertion of this leaf is 2 cm. from the top of the primary tumor at *T*.

Immediately above the primary tumor and under *B*, cross sections of the fresh stem showed the woody cylinder thickened a little on that side. The inner angle of the xylem wedges next the pith was greenish, but there was no conspicuous strand. Under the compound microscope two tumor strands are visible, however, in stained cross sections of this stem.

PETIOLE A.

(Plate LXXIV.)

Plate LXXIV, from slide 635 B 9, is a longitudinal section of petiole *A*, showing the tumor strand. This plate at its bottom joins on to the top of the next plate.

PETIOLE A.

(Plate LXXV.)

Plate LXXV, from slide 635 B 9, is a continuation of Plate LXXIV, showing the tumor strand expanding at bottom into a small tumor. For a continuation of this see next plate.

PETIOLE A.

(Plate LXXVI.)

The top of this plate, from slide 635 B 11, joins on to the bottom of the preceding plate, but the magnification is somewhat greater. The bulk of the plate (all of the center) is occupied by the tumor in which may be seen four centers of active proliferation.

DAISY NO. XIX.

WHOLE PLANT.

(Plate LXXVII.)

This plant was inoculated by needle pricks at *X*, *X* on January 13, 1911, and photographed April 15, 1911, three-fourths natural size. Diameter of stem at *Z*, 12 mm. Distance from the primary tumor to the insertion of *B*, 3.5 cm. and length of the secondary tumor in *B*, 9 cm. Stem structure at *S*, *S*. Portions of *A* and *B* were fixed in Carnoy's solution and also the stem to either side of 3. The stem between *A* and *B* was normal on the surface except for a slight swelling on the right side at the level of 3. Here on cross section a small green tumor strand in the wood was visible under the hand lens, but not in this manner at 1, 2, or 4.

CROSS SECTION OF STEM.

(Plate LXXVIII.)

Plate LXXVIII, from slide 595 A_1 8, gives a cross section of the right side of the stem not far from figure 3 of the preceding plate, showing a small tumor strand at *X* where the wood is slightly thickened. The remainder of the stem is normal. The bark was torn a little in mounting, and the outer portion of the pith was shriveled by the fixative. Section stained with methyl green and acid fuchsin. For appearance of the tumor strand more highly magnified and a little farther down (or up), see next plate.

CROSS SECTION OF STEM.

(Plate LXXIX.)

Plate LXXIX from slide 595 A_2 1 gives a cross section of the stem a few millimeters away from that shown on Plate LXXVIII and more highly magnified, showing a conspicuous tumor strand in the inner

wood near the pith. This is composed of large, soft, thin-walled cells, easily differentiated from the surrounding cells not only by their *form* but by their behavior toward stains, i. e., the surrounding cells are blue while these are red. Stain: methyl green and acid fuchsin.

PETIOLE A.

(Plate LXXX.)

Plate LXXX from slide 634 D 15 gives in longitudinal section an infected leaf trace at the base of petiole A, showing a conspicuous tumor strand with vessels to either side—spirals at the right. At *X* are cells of the tumor strand undergoing change into tracheids. Stained with methyl green and acid fuchsin. See plate IV for a longitudinal section through a normal leaf trace.

PETIOLE A.

(Plate LXXXI.)

Plate LXXXI from slide 634 A 4 shows a longitudinal section of petiole *A*, like Plate LXXX but farther up the leaf, i. e., the section was made between *Y* and *Y'*. To the left are nearly unchanged vessels of the leaf trace (stained blue in the section); to the right is the large-celled parenchyma of the ground tissue of the petiole. Between the two in the region of the spiral vessels is the tumor strand. Stain: Methyl green and acid fuchsin. Tracheids at *T'*, spirals at *S*, *S*, cambium at *C*, and phloem at *P*.

PETIOLE A.

(Plate LXXXII.)

Plate LXXXII, from slide 634 D 4, gives a longitudinal section through petiole *A* at the level of the unruptured secondary tumor *Y*, showing the stroma of the tumor, i. e., the development of tracheids in the tumor tissue. In the section the tracheids are blue and the tumor cells red. The normal direction of the tracheids is right and left, and in healthy leaf traces and also in the normal parts of diseased ones they are straight and parallel, as shown in the left-hand part of Plate LXXXI. In this section no wood fibers accompany the tracheids.

PETIOLE B.

(Plate LXXXIII.)

Plate LXXXIII, from slide 595 B_2 5, gives a cross section of petiole *B* to show its conversion into a stem under the influence of the tumor strand. The actual diameter of the section is 9 mm. The only unchanged parts of the petiole are the wings *W*, *W*, and a little coarse-celled parenchyma at the left. There is a slight gap

in the woody cylinder (bottom part above *W*), which is bridged over by tracheids which have grown at right angles to their normal direction. On the right side of the rapidly proliferating soft central mass of cells (tumor strand) are four whorls of tracheids inclosing soft cells (see next plate). The large cells to the right of these are portions of the petiole parenchyma surrounded by the tumor tissue and more or less modified by its presence. The dark spot in the upper part of this mass is a cell of the same type in the walls of which lignin has been deposited abnormally. Section stained with methyl green and acid fuchsin.

Whorls from Petiole B.

(Plate LXXXIV.)

Plate LXXXIV, from slide 595 B_2 8, gives a cross section of a small portion of petiole *B* at nearly the same level as Plate LXXXIII, showing the character of two of the tracheal whorls on the border of the tumor strand, which lies in the direction of the arrow. One of the whorls contains large cells and the other small ones. Section stained with methyl green and acid fuchsin.

Base of Petiole B.

(Plate LXXXV.)

Plate LXXXV, from slide 634 B 5, shows a cross section of the extreme base of petiole *B*. Seminormal wood wedges are at the left; abnormal ones at the right; tumor strand in the center. In the middle and on the left margin of this are crushed spiral vessels. Stained with methyl green and acid fuchsin.

DAISY NO. XX.

Whole Plant.

(Plate LXXXVI.)

This plant was inoculated January 13, 1911, by needle pricks at *X*, *X*, and photographed April 17, 1911, nearly natural size. Primary tumor at *XX*, beginning to decay; secondary tumors on leaves *A*, *B*, *C*, *D*, and *E*. Leaf *A* has shriveled nearly to its base, which is much swollen. Leaf *C*, which bore secondary tumors, was removed earlier for study and now bears a tumor on its cut curface. On *D* the tumor extends as far as *Y*. The distance from *Y* to insertion of *D* is 12 cm. The distance from *Z* to insertion of *E* is 10.5 cm., and from this point to the top of the primary tumor is an additional 2.5 cm.

The dissection notes are as follows: At 1, immediately under *B* (lower figure), the cross section shows a soft green tumor strand

at the junction of wood and pith, with slight enlargement of the wood on that side. The wood is also enlarged under *D*, and a tumor strand also apparently is present here, but not conspicuous; later (Pl. LXXXVII) it was demonstrated conclusively under the microscope. At 2 and 3 there was no distinct enlargement of the wood. Split longitudinally between 1 and 2 the stem showed to the naked eye a green strand narrowing upward and running out near 2.

Material was fixed from *D* and *E* and from the stem below *D*, all of which showed typical invasion.

Cross Section of Stem.

(Plate LXXXVII.)

Plate LXXXVII, from slide 632 C 3, gives a cross section of the stem below petiole *D*, showing a small tumor strand in the center. Below is pith; above are the spiral vessels of the inner wood, with medullary rays between them. At *X*, *X*, are two spiral vessels separated from their fellows and crushed.

Petiole D.

(Plates LXXXVIII to XC.)

Plate LXXXVIII, from slide 632 D 8, gives a longitudinal section of petiole *D*, showing the general relations of the small central neoplasm to the petiolar structure. A detail from the upper end of this tumor is shown on Plate LXXXIX, and a section near the periphery of the same tumor on Plate XC. Here the spirals are twisted and wedged apart. Tracheids occur to either side beyond the spirals and also in the tumor tissue at *X*. The nuclei, which appear to be in the spirals, are in tumor tissue under them.

Petiole D.

(Plate XCI.)

Plate XCI, from slide 632 D 10, gives a longitudinal section of petiole *D*, showing the appearance of one end of a small unruptured tumor developing from the tumor strand. The other end of the same tumor is shown in plate XCII. The two photographs together cover about three-fourths of the tumor.

Petiole D.

(Plate XCII.)

Plate XCII, from slide 632 D 10, shows a longitudinal section of petiole *D*. It was photographed from the other end of the tumor shown in the preceding plate. The wedged-off tracheids are at the

top. The normal tracheids and the spirals (somewhat displaced and crushed) are at the bottom. For continuation of the tumor strand see next plate (XCIII).

PETIOLE D.

(Plate XCIII.)

Plate XCIII, from slide 632 D 10, gives a longitudinal section of petiole *D*, taken one field away from the preceding plate (XCII), showing the continuation of the tumor strand (center) with vessels to either side.

DAISY NO. —.

MARGIN OF TUMOR IN PETIOLE.

(Plate XCIV.)

This petiole is from one of the plants inoculated January 13, 1911. It was fixed March 6, 1911. This plate, XCIV, from slide 574 A 4, shows the extreme margin of an unruptured secondary tumor, surrounded by and inclosing (*P*, *P*) normal large cells of the petiolar parenchyma. Rather large pieces of the material were fixed in Flemming's fixative *A* for 24 hours, then soaked 24 hours in a hardening fluid consisting of water 99, glacial acetic acid 0.7, and chromic acid 0.3 and washed in running water for 24 hours. The section was stained with methyl green and acid fuchsin. It belongs in this series, but no record was made of the plant number. At *T* is a bit of the stroma (a group of tracheids).

DAISY NO. XXXI.

FOLIAGE.

(Plates XCV to XCVII.)

Plate XCV shows the top of a plant inoculated April 26, 1911, in the leaves only by means of single needle pricks. It was photographed June 2, 1911, i. e., at the end of 37 days. Sections of two of these tumors for comparison with secondary tumors in the leaves follow as Plates XCVI and XCVII. The structure consists of a mixture of tracheids with soft-celled tumor tissue. There is no such differentiation of parts as shown in the secondary leaf tumors., i. e., no distinct stem structure.

HOP ON TOBACCO.

PITH OF STEM, CAMBIUM, ETC.

(Plates XCVIII to CIII.)

An effort was made to determine, by inoculation into young stems of rapidly growing tobacco plants, just which tissues were stimulated to proliferate. While the results from these inoculations are not

entirely conclusive, some very interesting phenomena were observed. It was shown, for instance, in material three weeks inoculated, that there was no proliferation along the needle track *in the pith* (Pl. XCVIII), but an area of proliferation developed in the vicinity of the cambium (Pl. XCIX). Small tumors arose also on the margins of the needle wounds at various levels. Plate C shows one at the mouth of the needle wound and Plate CI shows one about half way from the lips of the wound to the cambium line. Both of these small tumors are well provided with tracheids (*tr*), although they are both growing from the level of the bark parenchyma where no tracheids are normally present. The question here is whether the nodules were developed in place, i. e., out of infected parenchyma or from deeper cells whose progeny have come to the surface. A study of the whole series of sections would seem to indicate the former surmise as the correct one. The presence of tracheids and sieve tubes, *x*, also indicates this. Further studies will be made. In Plates C and CI the needle wound is at the right and the bark parenchyma at the left.

Perhaps the most interesting feature brought out by these inoculations relates to the tumor strand. In all of the daisy inoculations we have seen the tumor strand select the protoxylem region of the stem as the line of least resistance to its movements. In one of the tobacco plants a strand of small-celled tissue originating in the proliferations from one of the infected needle wounds passes through the middle of the coarse-celled bark parenchyma parallel to the surface of the stem, as shown in Plates CII and CIII, which join onto each other, *x* corresponding to *x*. The arrow indicates the direction of the surface of the stem, which is about 12 cell-layers away. The phloem lies at about an equal distance away from this strand in the opposite direction. This strand can be traced only on slides 23, 24, and 25. It ceases a little beyond the top of the first plate. Above and below these sections is the ordinary bark parenchyma. The strand is sparingly provided with tracheids, the smallest group being at x^1. The needle track lies just below the lower tracheids shown in Plate CIII.

DAISY TUMOR.

Chromium Reaction.

(Plate CIV.)

Figure 1 of Plate CIV shows the effect of five minutes' exposure to a hot saturated solution of potassium bichromate. The black pieces are slices of a young tumor; the pale ones are longitudinal sections and cross sections of normal young stems of the same plant.

Figure 2, right-hand side, shows the appearance of slices of young tumors exposed for five minutes to a hot saturated solution of potassium chromate. The left-hand colorless sections are pieces from the

same tumor which were extracted in hot ethyl alcohol for a few minutes before exposing to the hot bath of potassium chromate.

Cytological Studies.

(Plates CV, CVI, and CVII.)

Plate CV shows the first results of an attempt to photograph *Bact. tumefaciens* within the cells of the tumor. Lantern slides of these figures were exhibited at Philadelphia, April 4, 1912, before the American Association for Cancer Research. The nuclei are unstained, and counterstained with eosine. Rods and Y's are visible, also many negligible granules. The figures 1, 1 denote different levels in the same cell. The middle figure (branched rod) in the top row of Plate CVII is also from this field. In the lower right-hand figure a Y-body lies on the upper part of the nucleus—outside of it, however.

Plate CVI shows various levels in a single cell, which is nearly or quite free from precipitates and contains numerous bacteria (deep blue-black rods on a colorless ground). Its faint nucleus (*N*) may be seen at the bottom of the left-hand figure of the middle row. Above it in the upper left-hand part of the figure is the conspicuous nucleus of a cell which is free from infection, or nearly so. Owing to the slight penetration of the Zeiss oil immersion lens (3 mm. 1.30 n. a.) eight photomicrographs were made at different levels in this field so as to give as clear a picture as possible. The figures 1, 1; 2, 2; etc., denote corresponding places in the photographs. The bulbous ends of some of the rods, e. g., 2, are illusions due to the fact that only a portion of the rod is in sharp focus. The cell walls are unstained and invisible except with a very narrow pencil of rays.

Plate CVII shows additional rods and branched forms, and absence of these bodies in the nucleus. The numbered places represent slightly different levels in the same cells. The bacteria lie in such irregular positions that it is impossible to have many of them sharply defined at any one focus, e. g., in the lower part of the right-hand figure of the upper row are three rods in a chain, but the lower one is entirely out of focus. The one marked 4 in the lower figure shows distinct branching but had to be made a little vague to get 3. So, also, in the upper left-hand corner of the second figure from the bottom, 5 is distinctly branched but had to be thrown out to get portions of the three rods marked 6. The tumors used for these sections were impregnated in bulk and afterwards sectioned. In their preparation suitable material was sliced from the tumors and put for 24 hours into water containing 5 per cent gold chloride. It was then transferred to 0.25 per cent formic acid and kept in the dark for 24 hours (modification of Löwit's method for nerve fibers), after which it was washed, dehydrated, and embedded in paraffin in the usual way.

To bring out cell walls, protoplasm, and nucleus, some of the sections were subsequently stained on the slide with eosine, a faint stain proving most satisfactory.

(Plates CVIII and CIX.)

These two plates were made from photomicrographs of the 5μ thick section already referred to, which was stained by Gram, and washed in amyl alcohol until very pale. They are introduced to show lenticular chloroplasts which when seen edge-on might be confused with bacteria. The upper figure of Plate CVIII also shows a nucleus in amitotic division (see fig. 1 in text). The dark spot in the center of Plate CIX is a nucleus out of focus. To bring that into sharp focus threw the chloroplasts entirely out of focus.

ANALOGIES.

The higher plants are much simpler in structure and function than the higher animals and any comparison of the diseases of one with the other must take these facts into account. The plant is much closer to the soil than the animal, i. e., to the inorganic world.

Neither may the higher plants be regarded, like the higher animals, as units. They are rather to be regarded as congeries of such units, one or more of which may be destroyed without injury to the rest. A peach tree, for example, may be split longitudinally and, if care be exercised, the two halves will continue to live as separate trees, the circulation of the two parts being distinct because the movement of fluids, foods, etc., is up and down rather than sidewise. This is illustrated by the fact that if we cut all of the feeding roots on one side of a tree, the other side is not immediately injured, and, if we introduce into the circulation on one side of the trunk some readily diffusible substance, it quickly passes to the top of the tree on that side, but moves around the trunk to the other side only very slowly. If these overgrowths are cancers we might, therefore, expect what we find, namely, that the tree as a whole is less injured by such growths than would be an animal with a unitary structure and a rapid general blood circulation.

For similar reasons structural comparisons of the higher plants and animals is difficult. While such plants and animals are fundamentally alike in that both are composed of living cells multiplying in the same manner, capable of secretion and excretion, and having divided labors but united into a harmonious whole, yet when we come to compare their anatomy and the diverse ways in which the same physiological ends are accomplished, many difficulties arise. There is, for instance, in the plant no muscular system, no nervous system, and nothing corresponding to the complex digestive apparatus of the higher animals. The reproductive apparatus occu-

pies a smaller space in plants and is temporary. Also, to a much greater extent than in animals, the cells which secrete special products are distributed among other cells, rather than grouped together into special organs.

We might, therefore, expect to find in a plant cancer, if such a disease exists, both simplification, and combination of traits which in the animal appear to be peculiar to the tumors of special tissues.

When the writer compared crown-gall to sarcoma it was with such mental reservations as grow out of these differences. In the connective tissue of plants there is no interstitial substance, and therefore we could not expect to find it in a plant tumor derived from such tissues. Their general appearance, therefore, is more like a nontypical epithelioma or carcinoma; so also is the marked cell reaction in their vicinity, viz, increase of wood elements; and the structure of their secondary tumors. But, inasmuch as the round cells appear to be sometimes derivatives from the mother cells of medullary rays and show all gradations from actively vegetative unripe cells into well-developed ray cells forming overgrown medullary plates, and at other times are descendants of certain cells of the bark parenchyma, we may perhaps still regard them as resembling sarcomas. They are also like the latter in their predilection for young plants and the softer, more rapidly growing tissues of older plants, as well as in the luxuriant anaplastic character of their proliferations.

Another difficulty, however, has arisen. At that time I regarded the stroma of such tumors as ingrowths developed from the surrounding tissues under the stimulus of the tumor cells. Recent experiments undertaken to settle this point seem to indicate that the vessels (tracheids and sieve tubes) in part, at least, originate in the tumor directly from tumor cells; i. e., that the tumor-strand is a complex body containing some cells capable of originating vessels and others of a purely vegetative unripe sort.

The experiments leading to this conclusion were shallow pricks into stems. Here the needle did not reach to the phloem, much less to the still deeper cambium, and yet both sieve tubes and tracheids developed in these shallow tumors in tissue which never normally produces them.

In other words, if we have not misread the evidence, under the stimulus of this organism, certain cells of the bark parenchyma (upper part of Plate I) lose their specific features and become small rapidly proliferating purely vegetative (unripe) cells, while others develop tracheids and sieve tubes; i. e., tissues normally developed only by the deeper cambium. A study in serial section of small

tumors thus produced shows an abundant production of vessels in the absence of any wounding of the deeper cambium and in stems where no cork cambium has yet developed. The parenchyma cells of such tumors, as shown by their form, their size, and their behavior toward stains are purely vegetative and quite distinct from the matrix of bark parenchyma cells in which they lie and from some of which they have developed. This ability of cells of the bark parenchyma, which are as well differentiated as those shown in Plate I, to produce undifferentiated blastomous cells, some of which are capable of developing xylem and phloem, was not what we expected to find, but is clearly what the shallow inoculations seem to establish, and this conforms very well to a statement made in Bulletin 213, viz, "It is not yet beyond dispute that a cell mother of one kind can never give rise to a cell of another kind when a changed stimulus is applied."

In some cases a portion of the stroma seems to grow into the edges of the tumor from the surrounding tissue and the subject is so interesting that further studies will be made.

The tumor development is not due to diminished external resistance but to an increased internal stimulus central within certain infected cells. That this stimulus can act at a distance—i. e., is not confined strictly to the infected cells—seems probable both from the macroscopic appearance of stems penetrated by the tumor strand and from the findings in the sections made from the material treated with gold chloride. The reader is referred especially to the wood overgrowths shown on Plates XXV, LXII (Figure A), and LXIII. Here the tumor strand containing the bacteria lies in previously formed dormant tissue at the base of the wood wedges next to the pith far away from the cambium which originates new wood. Yet the wood on that side of the stem is enormously overgrown. Since we have no reason to think the cambium infected by the tumor-producing bacteria because it has developed normally and is only different from that on the other side of the stem in having laid down an excessive volume of wood, we can only account for this overgrowth by postulating action at a distance, exerted by the tumor strand. We might assume either a weak toxic action on the cambium exerted by substances diffused from the infected cells of the tumor strand, or only that the growth has been induced by the stimulus of an extra supply of water and other foods drawn into this part of the stem by the presence of the rapidly growing soft tissue of the strand. In that case, however, it is hard to account for the fact that the bark is not involved in this overgrowth. The amount of overgrowth in the wood seems to depend on the size of the tumor strand. Compare in this particular the plates already referred to with Plates LXVII and LXXVIII, in which the tumor strand is small.

Reference has been made (p. 12) to the morphological similarities between this disease and malignant growths in animals. Here some of the physiological resemblances may be mentioned:

(1) The disease is not an abscess, but an abnormal organization process.

(2) The growth is extra-physiological and detrimental to the plant.

(3) The growth tends to return after excision.

(4) It tends to develop in wounds or irritated places.

(5) There are grades of virulence.

(6) The structure of the gall is looser than that of normal tissues, and decay often sets in early, forming open wounds subject to secondary infections.

(7) The nuclei are hyperchromatic and often stain deepest on the edges of the growth, as in cancer.

(8) There occur in excess in the morbid tissues certain cell products (chemical substances) serving to distinguish the anaplastic cells from normal meristematic or embryonic tissues.

(9) In some cases increased resistance has been developed by inoculations.

Adami in his Principles of Pathology (Vol. I, p. 651) defines neoplastic tumors as follows:

"It is this autonomy, this growth independent of function and of either present or future needs of the organism in which they occur and from which they gain their nourishment, independent also of obvious stimulation from without, that distinguishes the neoplasms proper from all other forms of tissue growth."

From the same source (p. 652) several other pertinent definitions may be quoted. Wishing to include teratomas, he says:

"We prefer C. P. White's statement that 'a tumor proper is a mass of cells, tissues, or organs resembling those normally present, but arranged atypically. It grows at the expense of the organism without at the same time subserving any useful function.' Von Rindfleisch characterizes them as a 'localized degenerative excess of growth'; i. e., the very excess of growth is regarded as in itself a degeneration; Birch-Hirschfeld, as originating spontaneously, becoming separate from the physiological tissues in their physiological and functional relationships, as developing from the cells of the body, and possessing progressive growth; Ribbert, as 'self-confined, dependent upon the organism for their nourishment, but otherwise largely if not quite independent, corresponding more or less but never absolutely with the tissues of the natural body, and presenting no definite limit, to their growth.' Lubarsch's definition is closely allied: 'Under tumor proper we have to understand those growths of apparently independent origin which histologically correspond in structure more or

less completely with the matrix from which they originate, but in form are atypical; which further, in spite of their organic connection with that matrix, and in subjection apparently to laws of their own, pursue an independent existence which is not, or only exceptionally, of advantage to the organism as a whole.'"

Excluding the hypotheses, these definitions apply strictly to the growths here described. Indeed, in terms of morphology and physiology, excluding teratomas, it would be difficult to frame a definition of tumors as a whole which would exclude crown galls. The only way they can be excluded is to say that they occur on plants and are of known origin, whereas the others occur on animals and are of unknown origin, and that would be begging the question.

RÉSUMÉ.

The principal facts brought to light during this study and our earlier studies may be summarized as follows:

(1) Crown galls occur on a great variety of plants, but not always on the crown; any part of the root or shoot is liable to attack.

(2) They are injurious to the plant in varying degrees, depending on the species, on the parts attacked, on the size and vigor of the individual, etc. They are most injurious to young and rapidly growing plants.

(3) Young, well-nourished, rapidly-growing tissues take the disease more readily than old or slow-growing ones.

(4) They are all of parasitic origin, unless the one on the beet studied by Jensen, Reinelt, and Spisar, in Europe, should prove an exception. We found it difficult to obtain virulent cultures from old galls occurring naturally on the sugar beet, but did finally obtain slow-growing tumors from certain colonies (Bul. 213, Pl. XXXVI).

(5) The structure of crown gall is unlike that of club-root of cabbage, which is a hypertrophy rather than a hyperplasia.

(6) We have isolated the parasite from 24 species belonging to 14 families of phanerogams. Some species have resisted infection.

(7) These galls are due to schizomycetes, either to one polymorphic species, or to several closely related species. Further studies are necessary. For notes on the morphology and biology of these isolations see Bulletin 213, page 127.

(8) The infectious nature of the organism isolated has been proved by hundreds of inoculations and its ability to produce galls on other plants than the one from which it was isolated by many cross inoculations. (Bul. 213, p. 133.)

(9) The parasite has been shown to occur not only in the primary tumor, but also in the secondary tumors and in the connecting tumor strand. Once only in the latter.

(10) Various noninfectious saprophytes also occur in crown galls especially when old, viz, white, green-fluorescent, yellow, and pink bacteria; fungi; mites; myxomycetes, etc. Other infectious organisms may also gain an entrance, viz, the pear-blight bacillus, the fungi of root rot, and borers which, especially in the peach, seem to prefer the soft tissues of the galls.

(11) The parasite has been grown in pure culture on a variety of media and its morphology and cultural peculiarities determined.

(12) When taken from young agar or bouillon cultures, *Bact. tumefaciens* is a short rod with rounded ends, dividing by fission and motile by means of a polar flagellum (sometimes 2 or 3 are present). Short chains and filaments occur. Under unfavorable conditions branched forms (involution bodies) are common. It stains readily, but not by Gram. It is not acid-fast. It is not distinctly capsulate and does not produce spores. For additional details see Bulletin 213.

(13) It grows readily on a variety of the common culture-media, but nearly always it is slow to start off when cultivated directly from the tumors. It forms small, white, wet-glistening, circular, flat colonies on agar plates and is also white on other media. It does not liquefy gelatin nor are its gelatin colonies characteristic. The organism is aerobic in its tendencies. It forms stringy filamentous growths in bouillon. The coagulation of milk is delayed. It blues litmus milk. It does not reduce nitrates nor grow well in Cohn's solution (daisy). It is sensitive to heat, to dry air, to acids, and to germicides. For additional notes see Bulletin 213.

(14) The organism slowly loses virulence when grown on culture-media. We believe that many of the bacteria also lose virulence within the tumor, because not all colonies growing typically on agar poured plates, and in other media, are infectious.

(15) Some of its biochemical properties are now known, to wit, the production from grape sugar of an acid which seems to play an important rôle in the tumor development. Alcohol also occurs.

(16) It has also been stained within the tissues of the tumor and its form and locus therein determined.

(17) The morphology and biological peculiarities of the tumor growth have been studied.

(18) The tissues of the gall multiply excessively and in opposition to the best interests of the plant.

(19) The galled tissue, which is often of a soft, fleshy nature, is much subject to decay. It is not usually corked over, and this absence of a protective surface allows the ready entrance of water and of other parasites.

(20) The tumor originates in meristem, usually in the cambium region. It may perish within a few months or continue to grow (parts of it) for years.

(21) The tumor consists, or may consist, not only of parenchyma cells but also of vessels and fibers, i. e., it is provided with a stroma which develops gradually as the tumor grows. A proliferating tumor usually contains not only meristem but pitted vessels and sieve tubes; it may also contain wood fibers, but does not always.

(22) The tumor sends out roots (tumor strands) into the normal tissues. These may extend for some distance from the tumor—how far is not known. These strands consist of meristem capable of originating medullary rays, tracheids, and sieve tubes. In the daisy the strand passes through the protoxylem region of the stem. It is rich in chloroplasts. It usually takes a deeper stain than the surrounding tissues, from which it is sharply delimited. A considerable part of it consists of unripe, actively vegetating cells.

(23) In the daisy the infiltrations are not through the vessels, but between them in a tissue offering little resistance to intrusion, i. e., the region occupied by the thin-walled, delicate spiral vessels.

(24) In the substance of these deep-lying strands secondary tumors develop. These gradually rupture their way to the surface.

(25) The secondary tumors tend to take on the structure of the primary tumor, e. g., if the latter is in the stem and the former in a leaf, the secondary tumor shows a stem structure.

(26) The stimulus to tumor development comes from the presence of the parasite within certain of the cells. Apparently it is not in all. The organism has not been observed with certainty outside of the cells, either in the vessels or the intercellular spaces, nor is it abundant in the cells. Usually copious inoculations have to be made to insure cultures.

(27) Under the microscope it can not be made out in unstained sections with any certainty, and most bacterial stains also fail to differentiate it in the tissues. (Histological Pls. VII to CIII.) It is best observed in tissues impregnated with chloride of gold. (Pls. CV to CVII.)

(28) When subjected to unfavorable conditions in cultures the parasite develops involution forms consisting of club-shaped, Y-shaped, and variously branched bodies. The same bodies occur within the cells of the tumor, making it reasonable to infer that the parasite is there exposed to similar unfavorable conditions.

(29) These involution forms may be produced at will by the addition of dilute acid to young cultures. The abnormal forms (Y's, etc.) thus produced either refuse to grow when sown in agar plates or develop colonies slowly. The same results are obtained very often on making poured plates from the tumors, viz, either no colonies appear or slowly developing ones, but subcultures from these slowly developing colonies grow promptly. Sometimes also from the tumor one gets the organism promptly on agar poured plates (third

day). In the delayed cases the mere change from tumor tissue to culture media is not the cause of the delay.

(30) By repeated inoculations through a series of years we obtained (Bul. 213, p. 177) plants which appeared to be more resistant to the disease than check plants, but by subsequent inoculations on descendants of these plants we obtained numerous well-developed primary and secondary tumors, so that the resistance which we obtained must be regarded either (*a*) as of a fugitive nature, or (*b*) as of a low grade easily overcome by a more virulent strain of the parasite. That the cultures used for these subsequent inoculations came from a more virulent strain may be assumed, we think, both because they were plated from a tumor which appeared on one of our most resistant plants, and because the cultures tried on a great number of plants (including those described in this bulletin) produced primary tumors very quickly and showed an unusually strong tendency to develop secondary tumors.

(31) The relation between the host and the parasite may be regarded as a symbiosis in which the parasite has the advantage.

(32) The bacterium is a soil organism and planters should aim to keep their lands free from it by refusing to plant infected stock.

(33) Nurserymen should plant on uninfected land and carefully avoid heeling good stock into soil which has previously received infected plants. Nurserymen have been largely responsible for the dissemination of this disease.

(34) The organism is a wound parasite. Its entrance is favored by careless grafting (Hedgcock) and by the presence of borers, nematodes, etc.

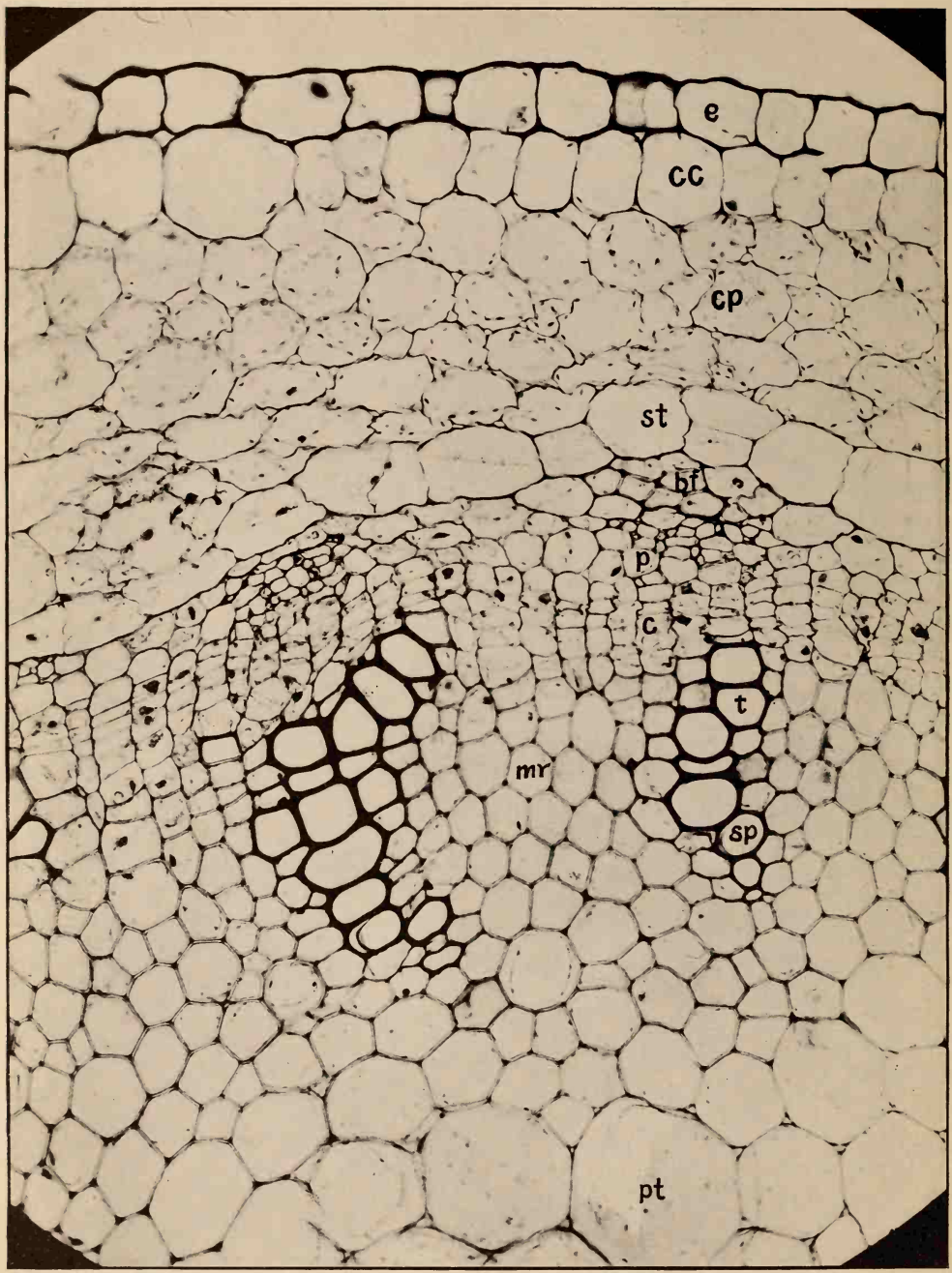
(35) These galls occur on the roots of Legumes and have been mistaken for the nitrogen root nodules.

(36) The development of this disease is regarded as closely paralleling what takes place in cancer of men and animals.

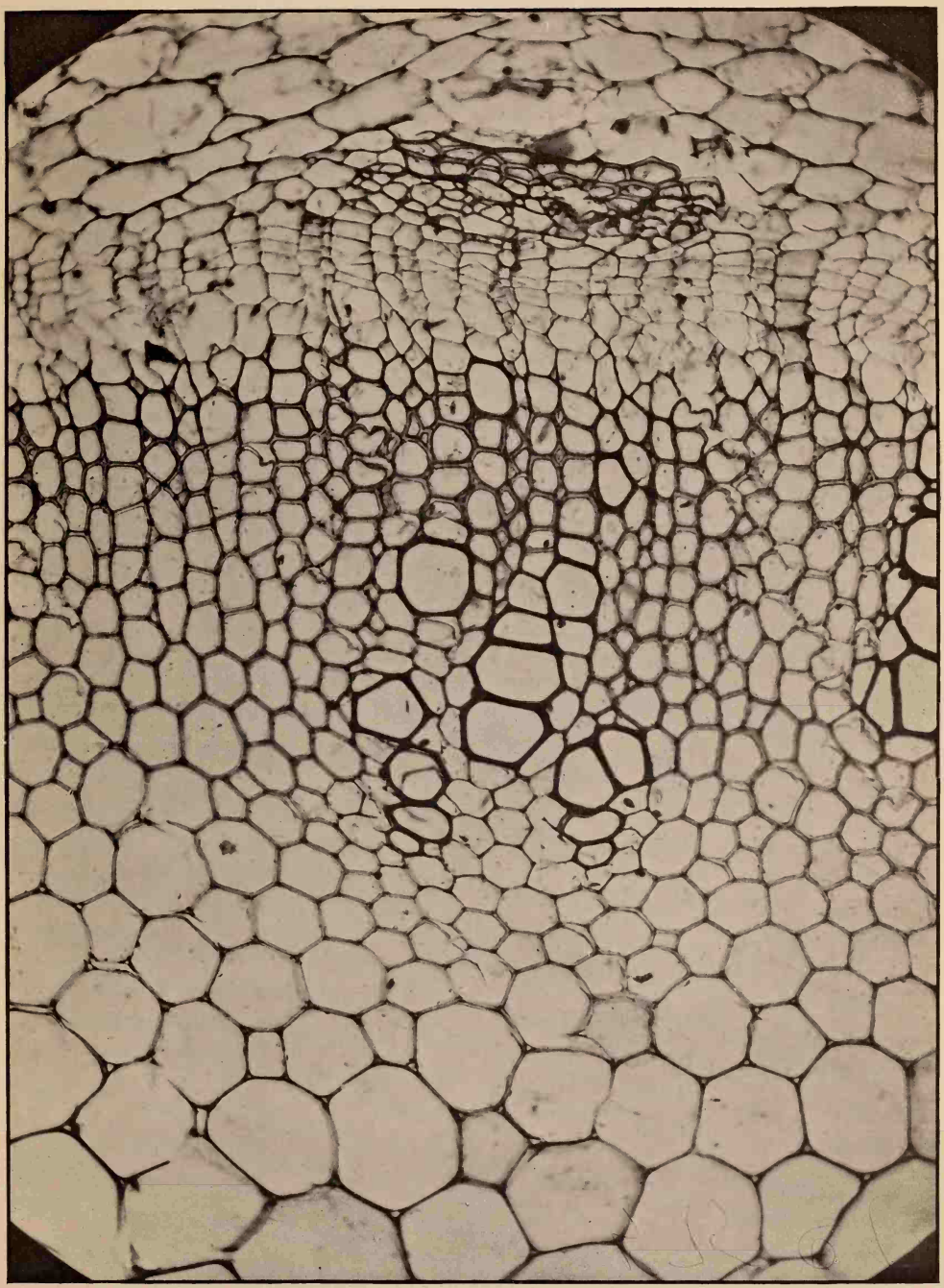
(37) There are no true metastases in crown gall, but this does not, to our mind, militate against the comparison, for whether a cancer shall be propagated by floating islands of tissue, or only by tumor-strands, appears to be a secondary matter depending on the character of the host tissues rather than on the nature of the disease. The essential element is the internal stimulus to cell division.

(38) Nothing in this bulletin should be construed as indicating that we think the organism causing crown galls is able also to cause human cancer, but only that we believe the latter due to a cell parasite of some sort, and offer the preceding pages in support of this contention.

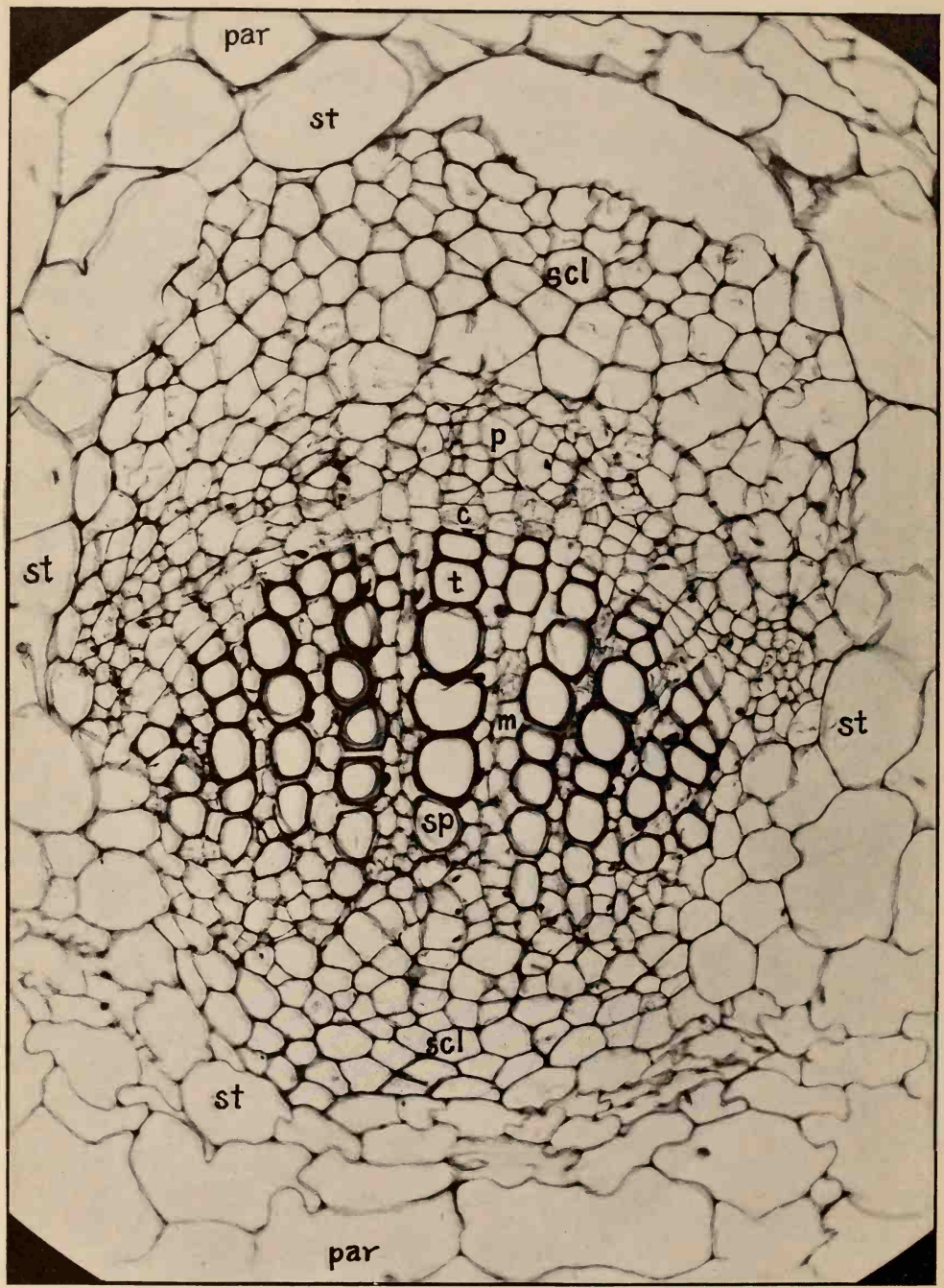
PLATES.



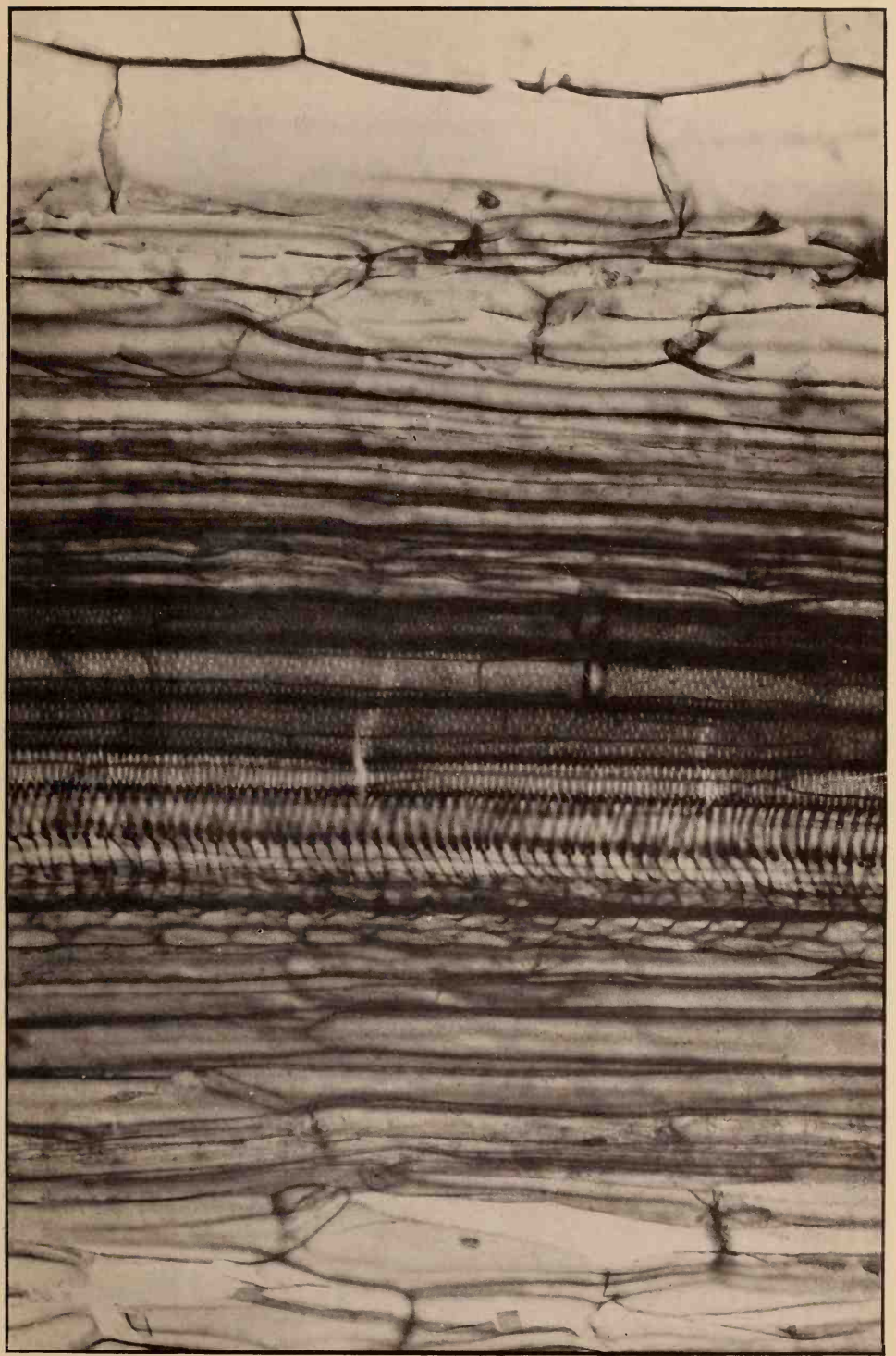
Daisy 1. Cross section of a normal young branch in the outer region of which, i. e., between st and e, shallow needle pricks may produce tumors containing tracheids (p. 25).



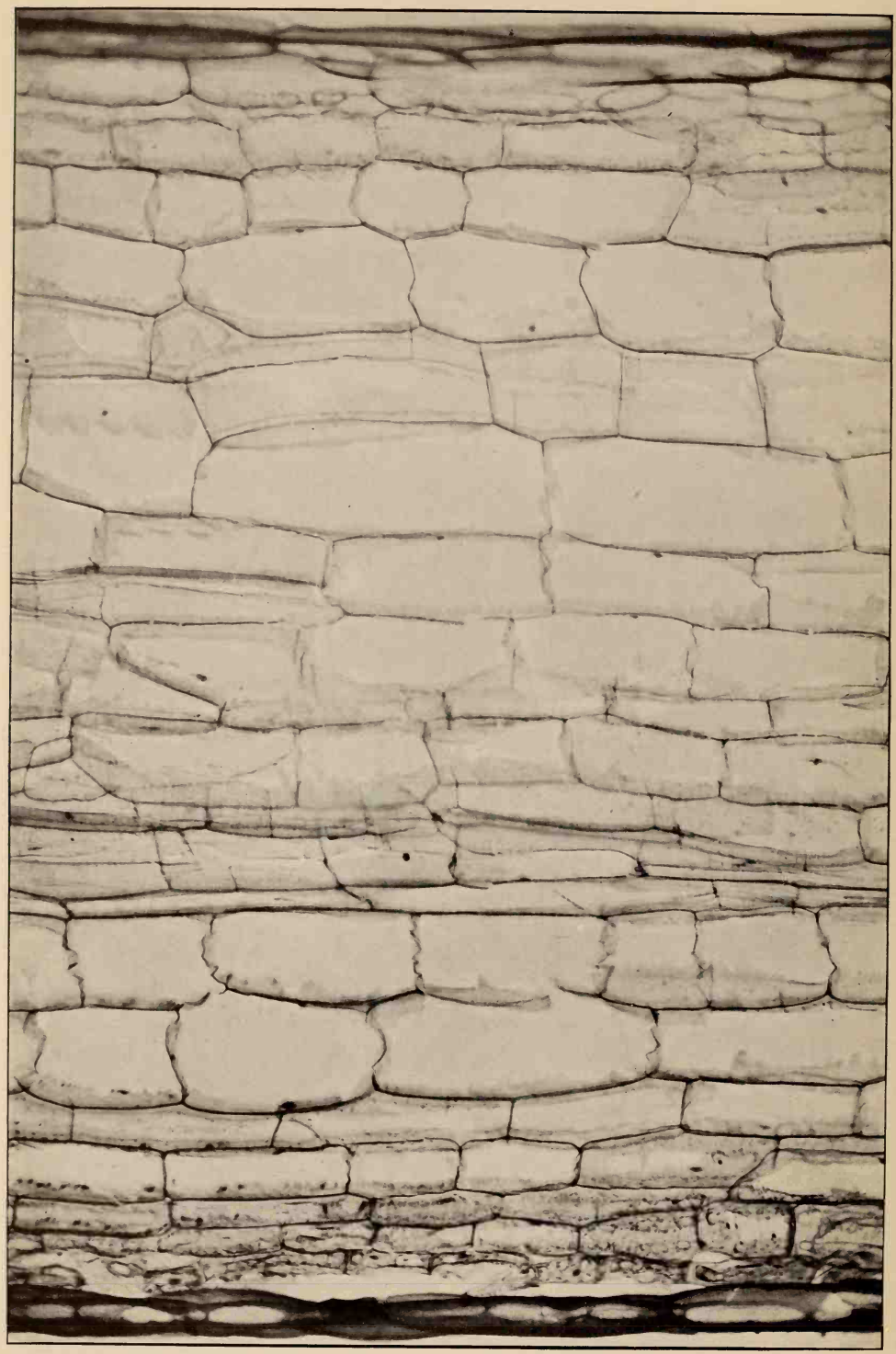
Daisy I. Cross section of normal branch somewhat older than the preceding (p. 25).



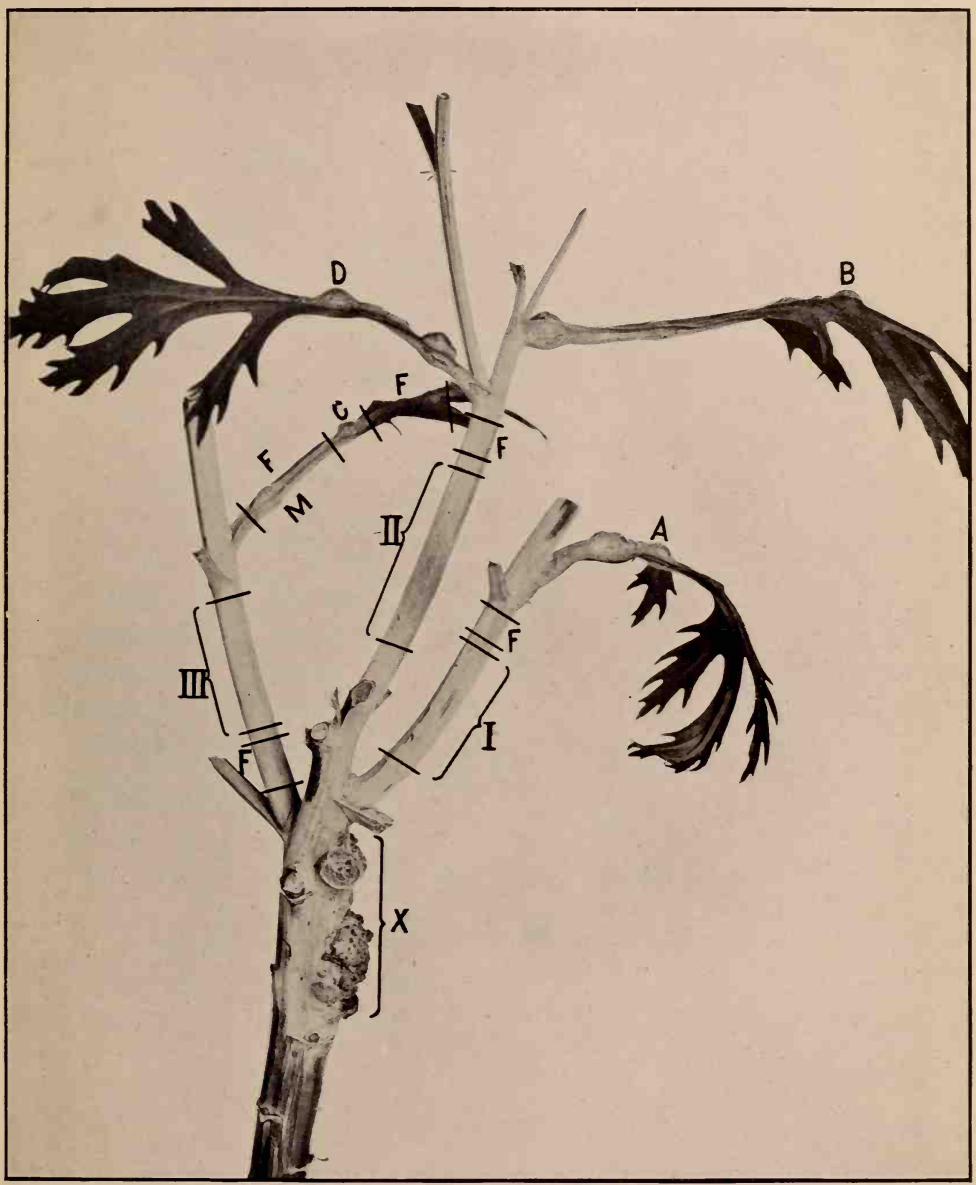
Daisy XIV. Cross section of a normal leaf-trace (p. 26), showing unilateral structure.



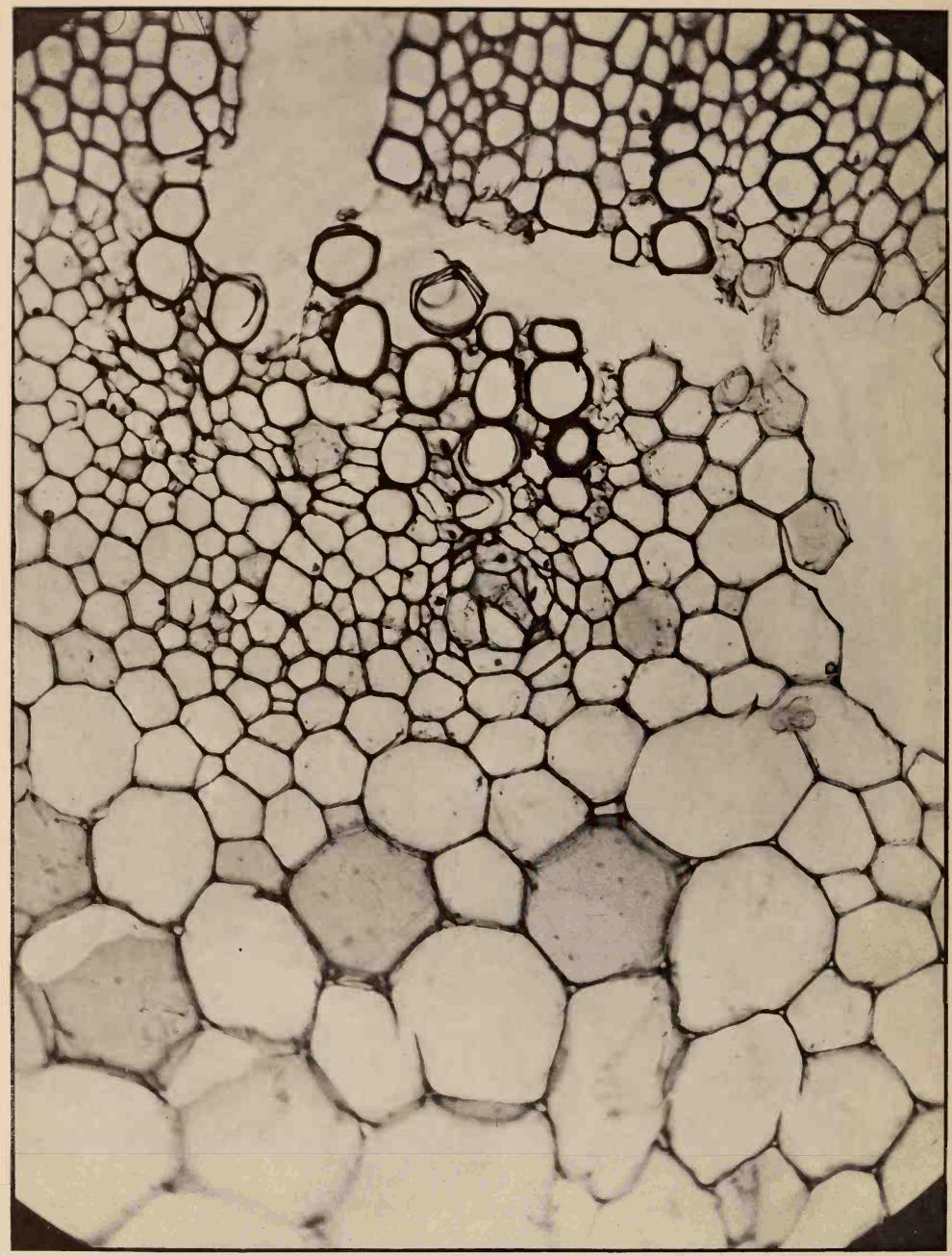
Daisy XXX. Longitudinal section of a normal leaf-trace (p. 26).



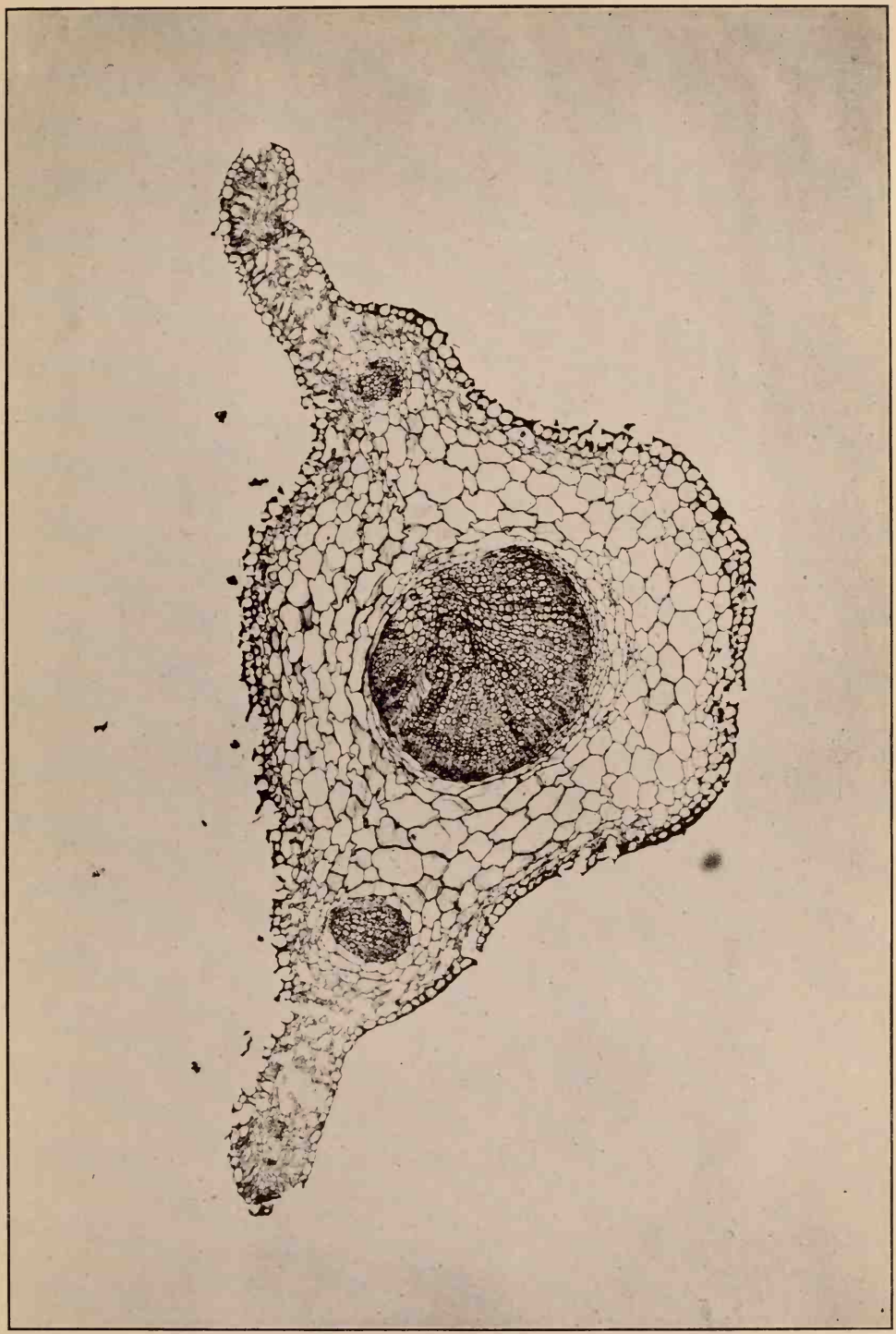
Daisy XXX. Longitudinal section of a normal petiole between leaf-traces (p. 26).



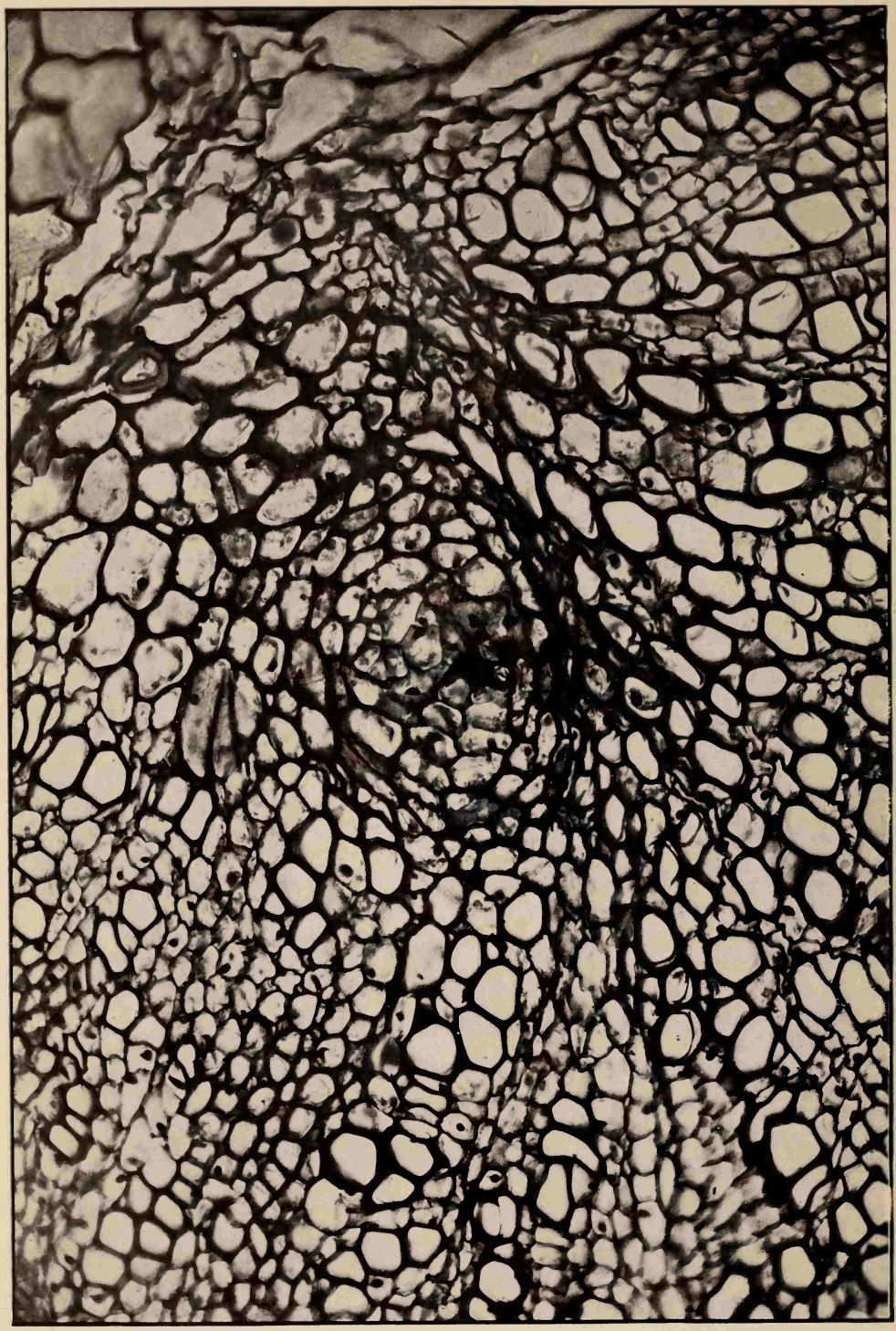
Daisy I. Part of plant showing primary stem tumors (at X) and secondary leaf tumors (p. 26).



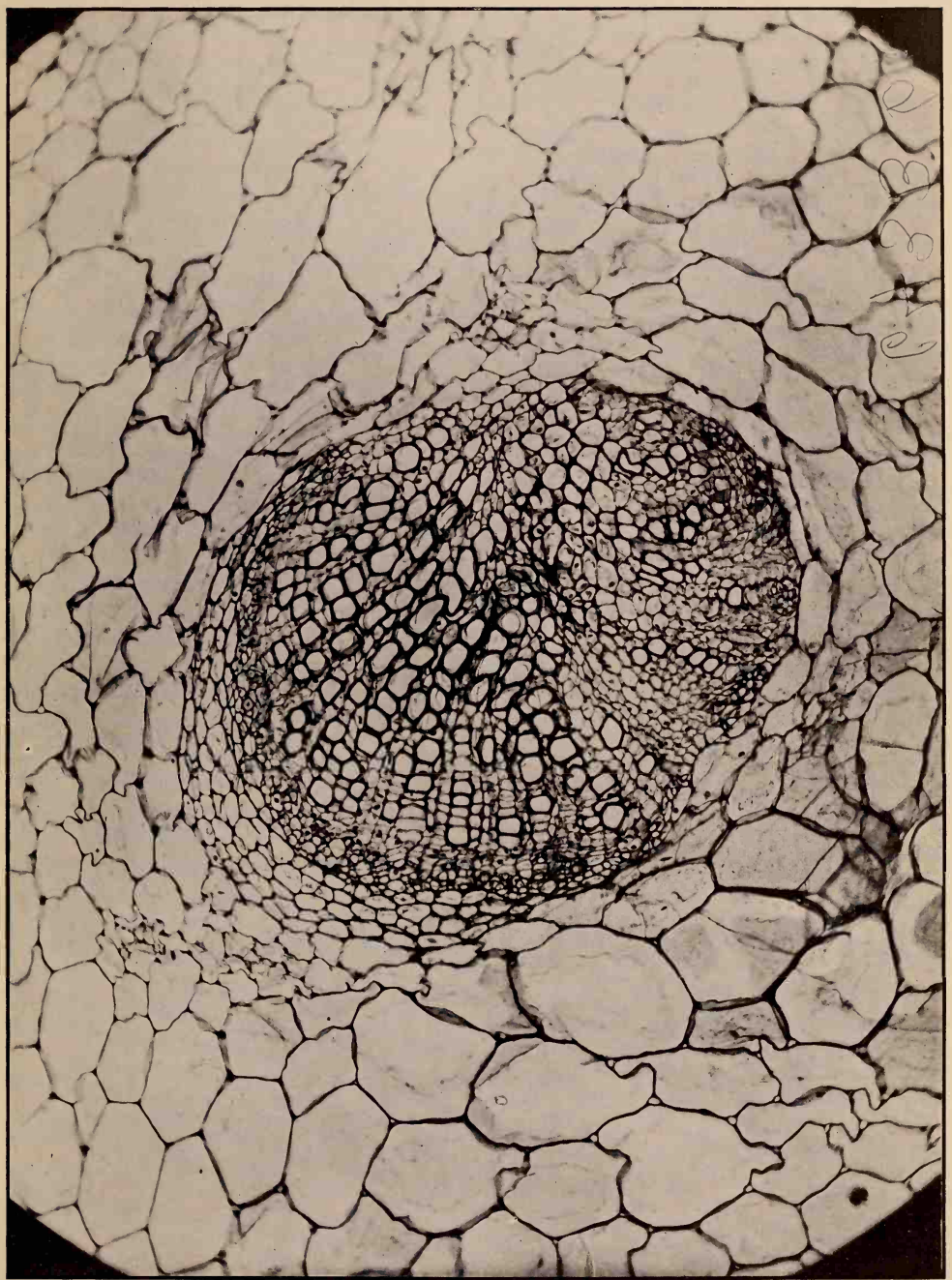
Daisy I. Cross section of Branch III between tumors, showing tumor-strand which passes into petiole C (p. 27).



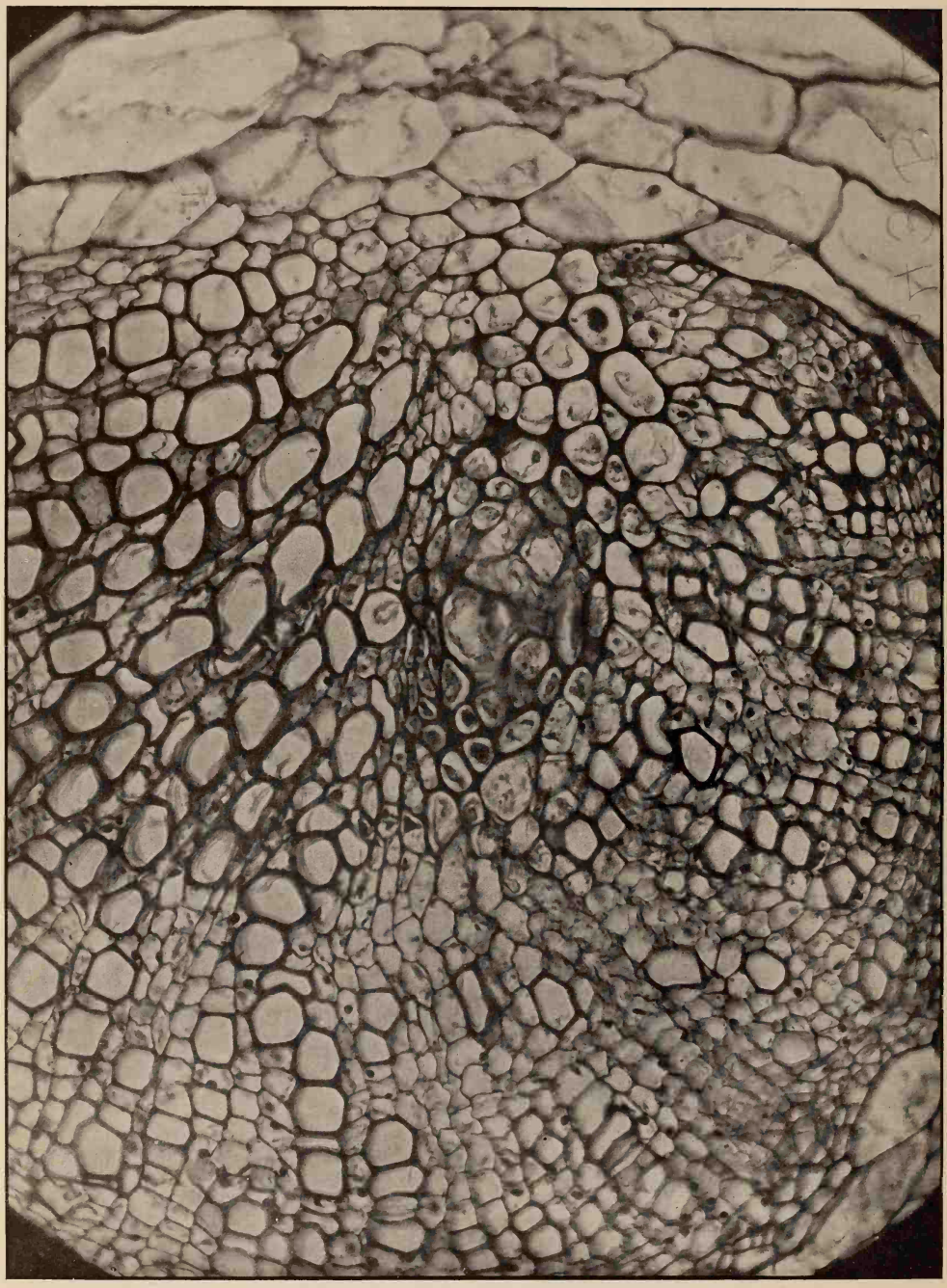
Daisy I. Cross section of petiole C, central leaf trace diseased (p. 27).



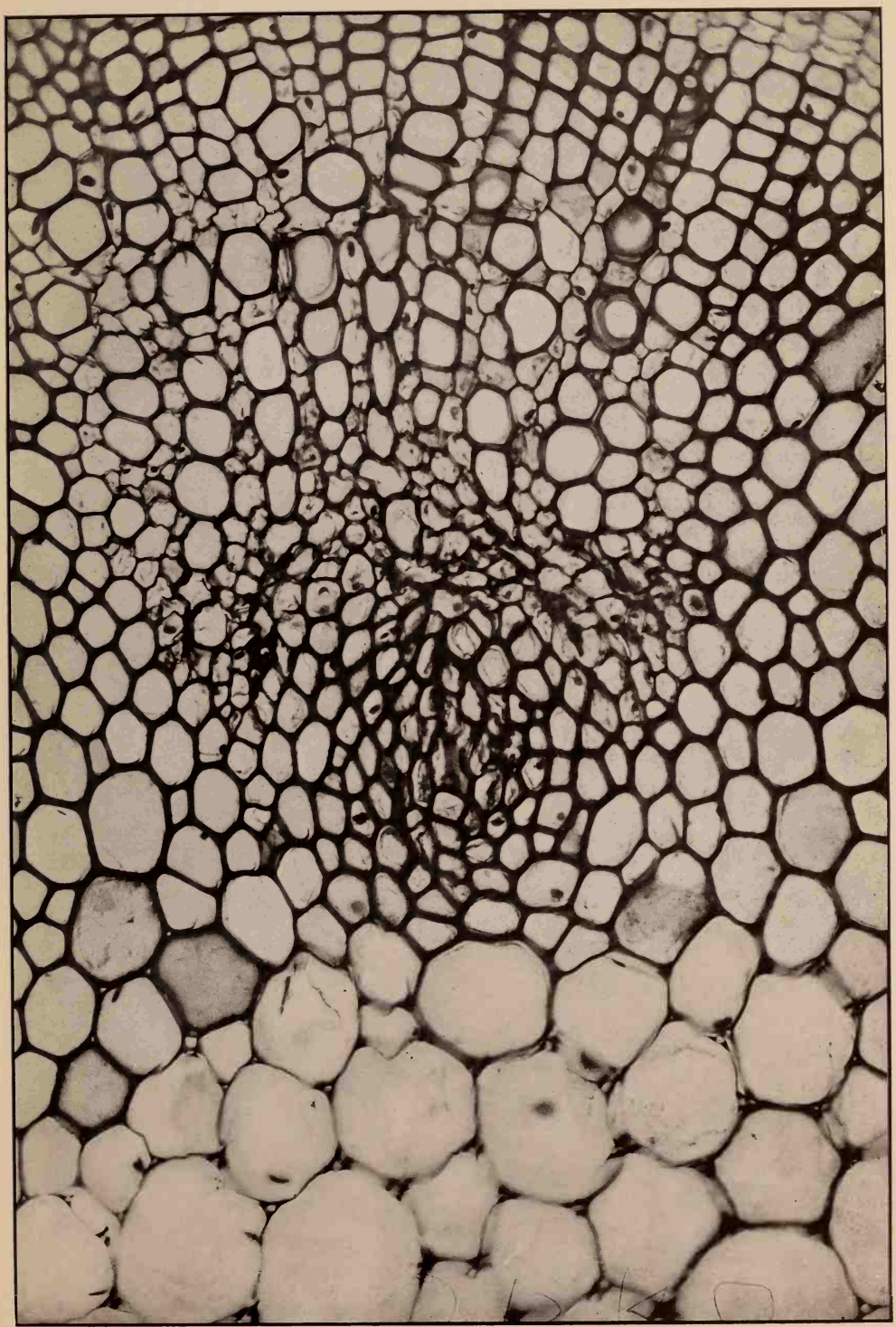
Daisy I. Center of tumor in petiole C, showing the tumor-strand (p. 27).



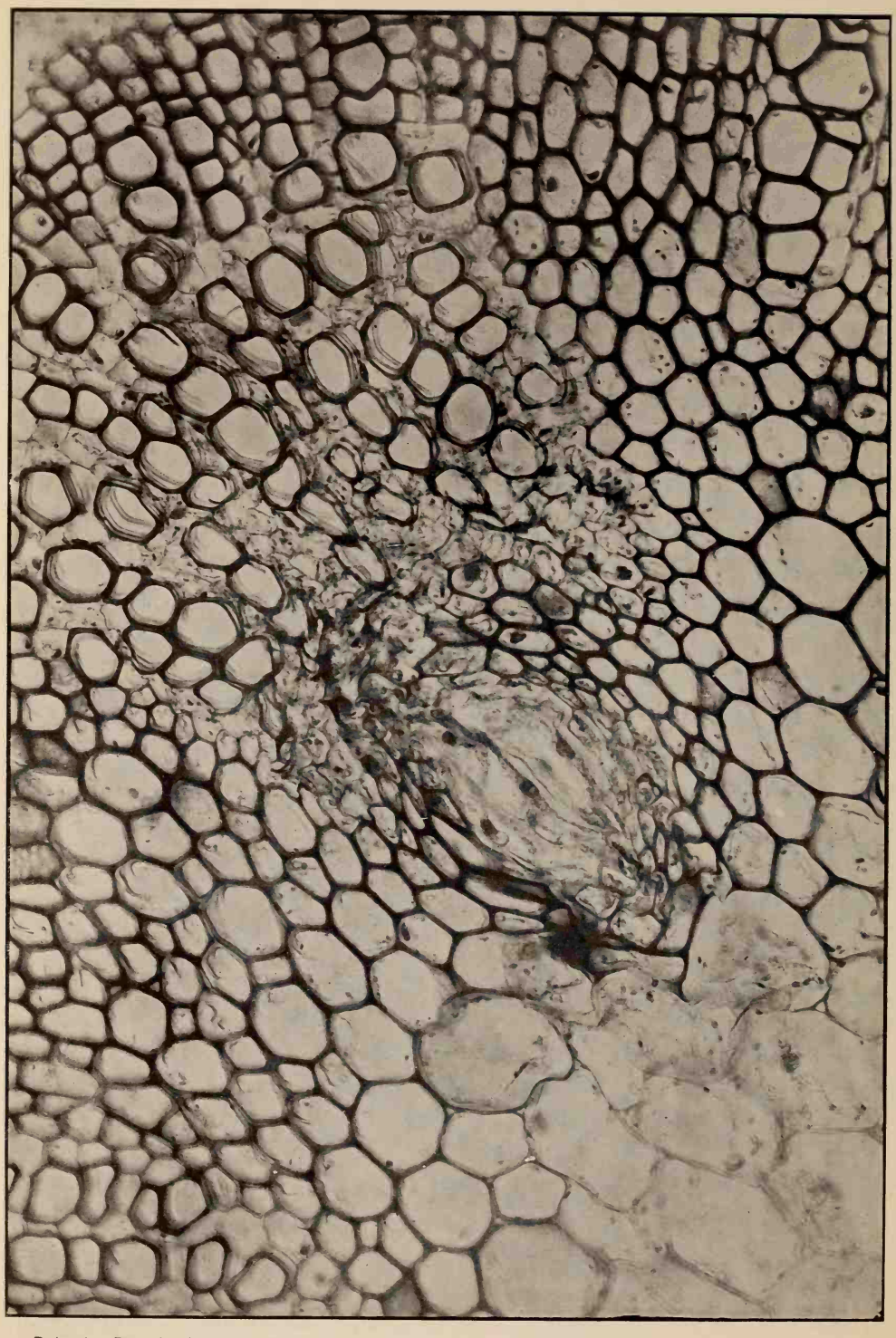
Daisy I. Cross section of petiole C at another level, showing conversion of leaf-trace into a secondary tumor (p. 27).



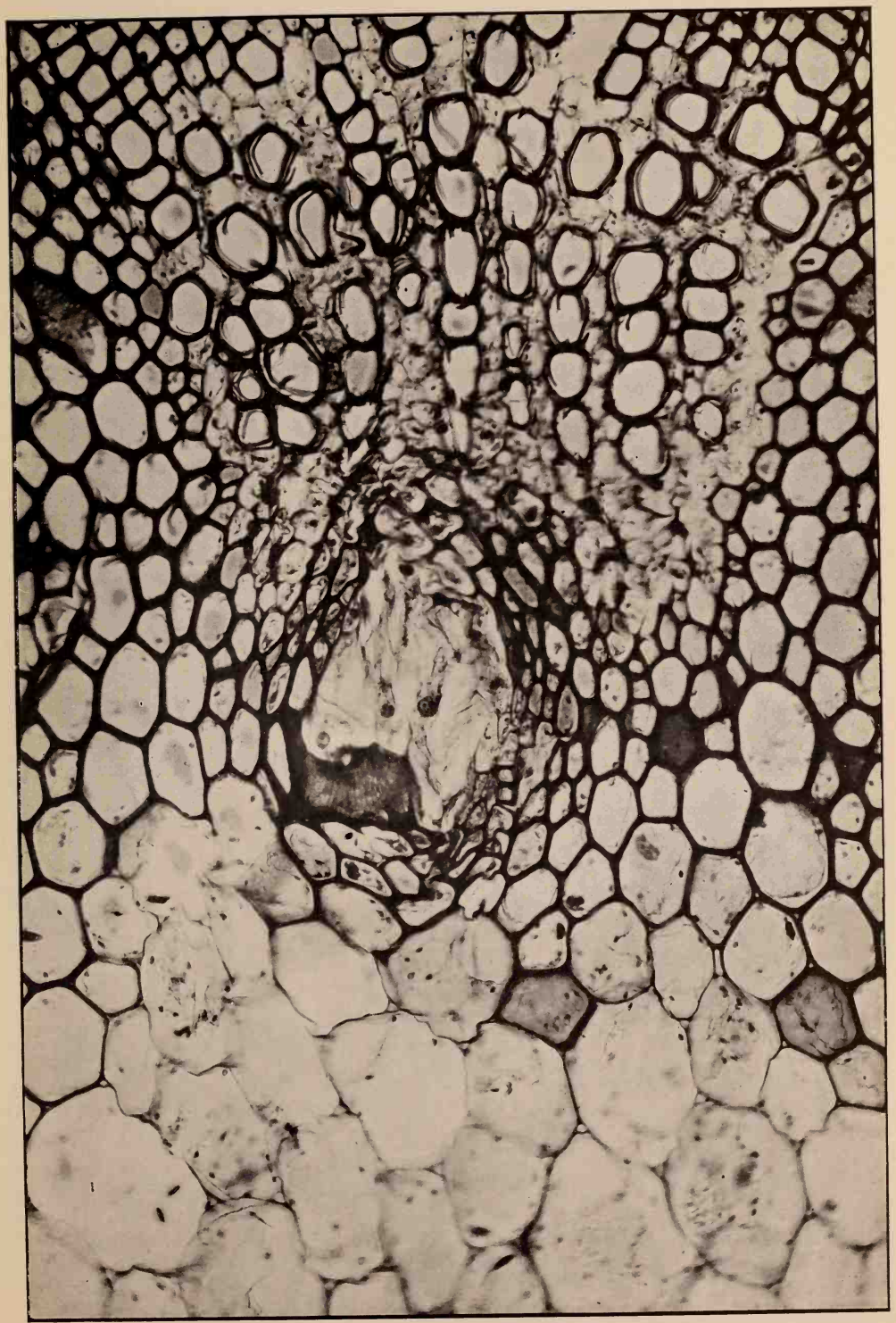
Daisy I. Same as Plate X, but center of tumor, showing strand in its middle portion (p. 27).



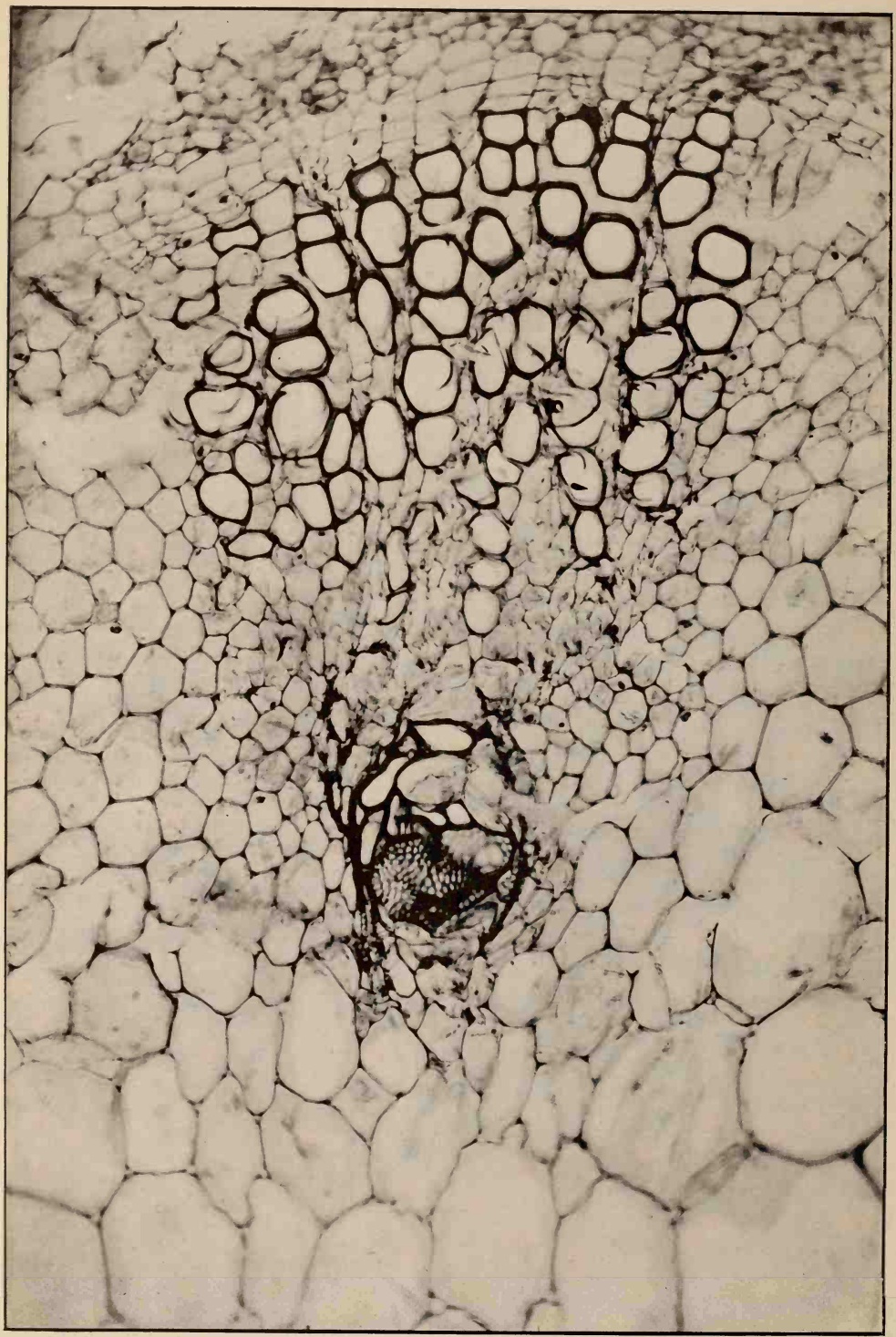
Daisy I. Branch I in cross section between tumors, showing small-celled tumor-strand (p. 28).



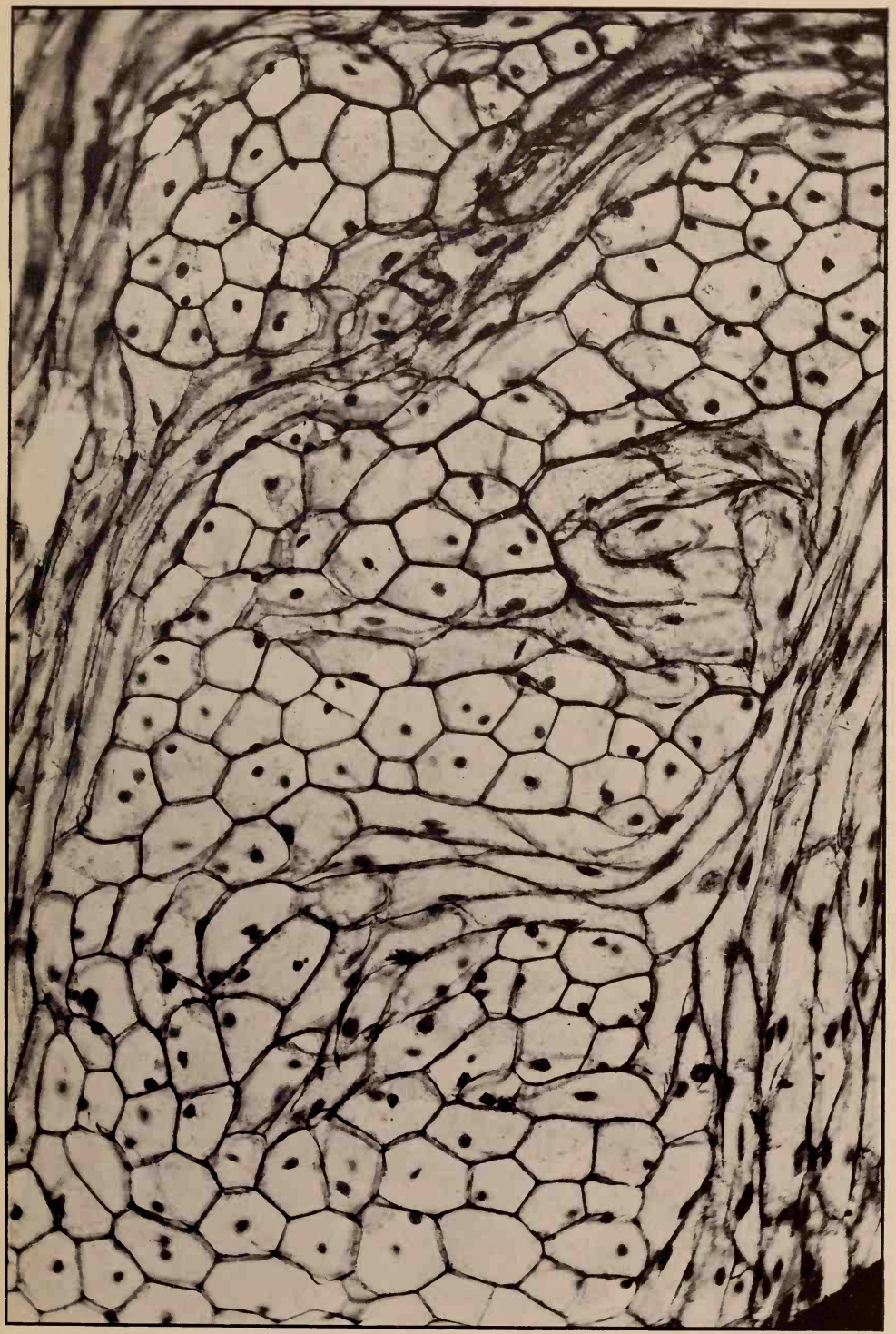
Daisy I. Branch I in cross section at another level, showing large, soft cells in the tumor-strand (p. 28).



Daisy I. Cross section of Branch I, showing tracheids developing in the tumor-strand (p. 28).

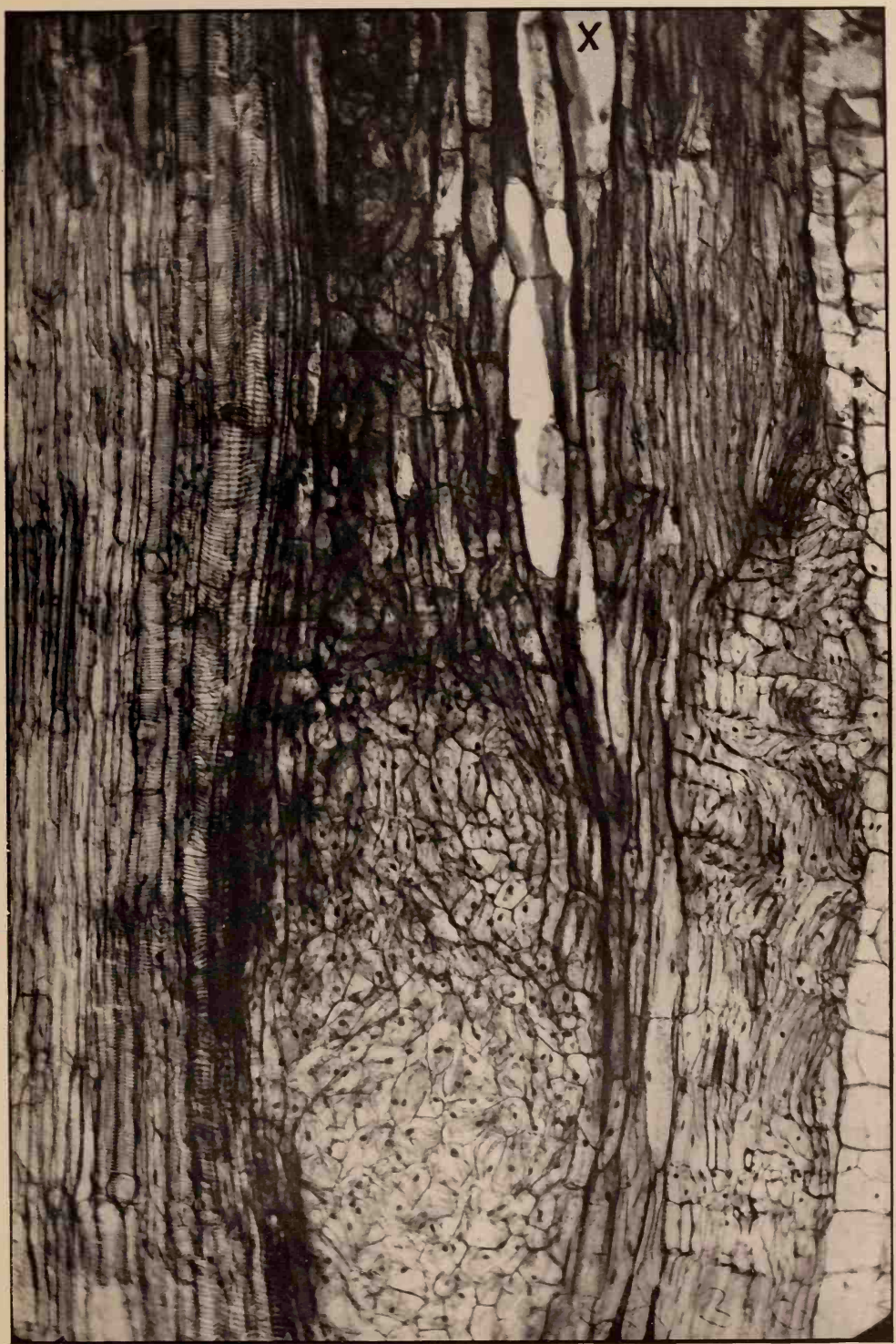


Daisy I. Branch II in cross section between tumors; tracheids in strand (p. 28).

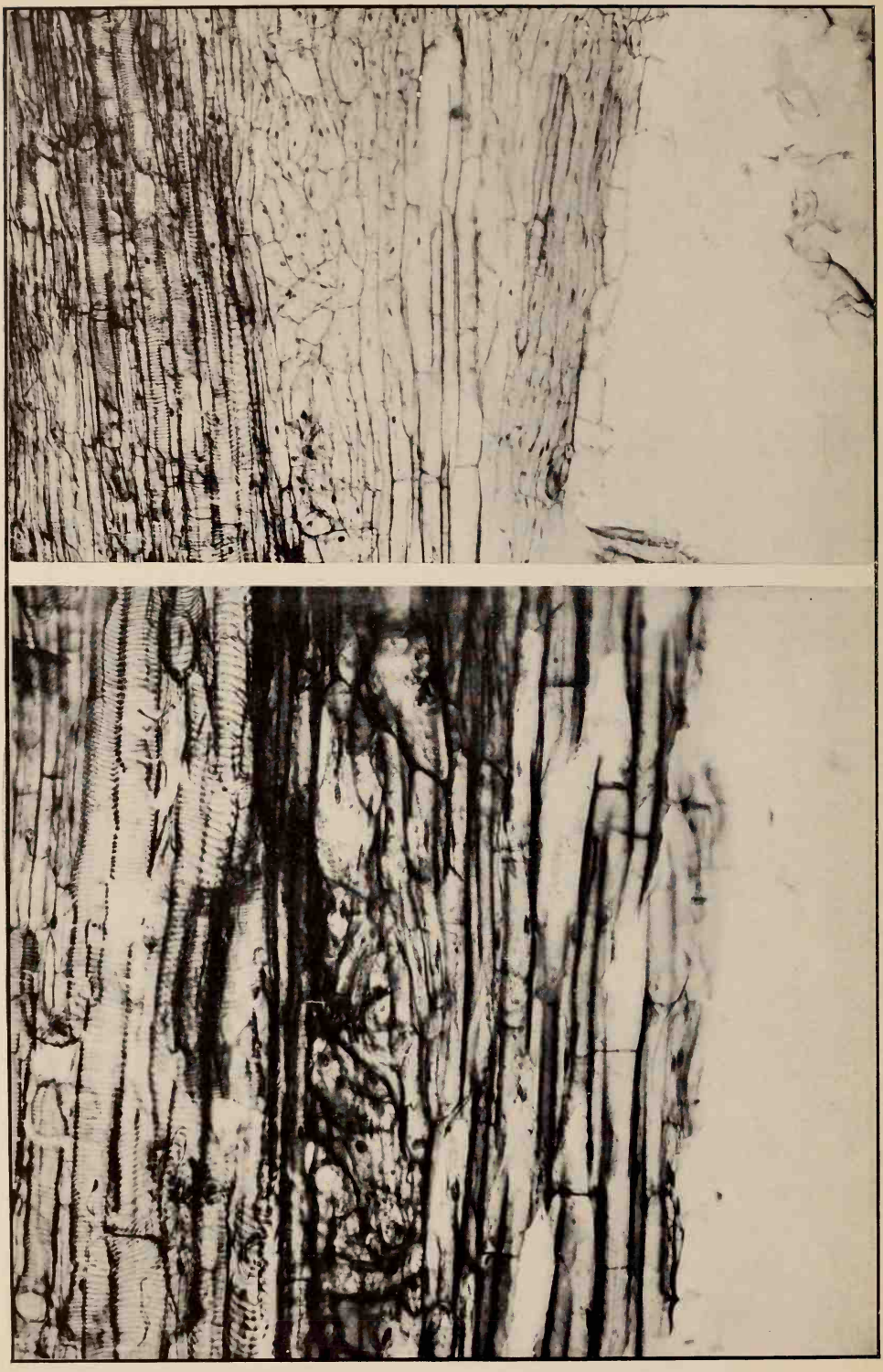


Daisy I. Petiole C. Tumor tissue with bi-nucleate cells (p. 28).

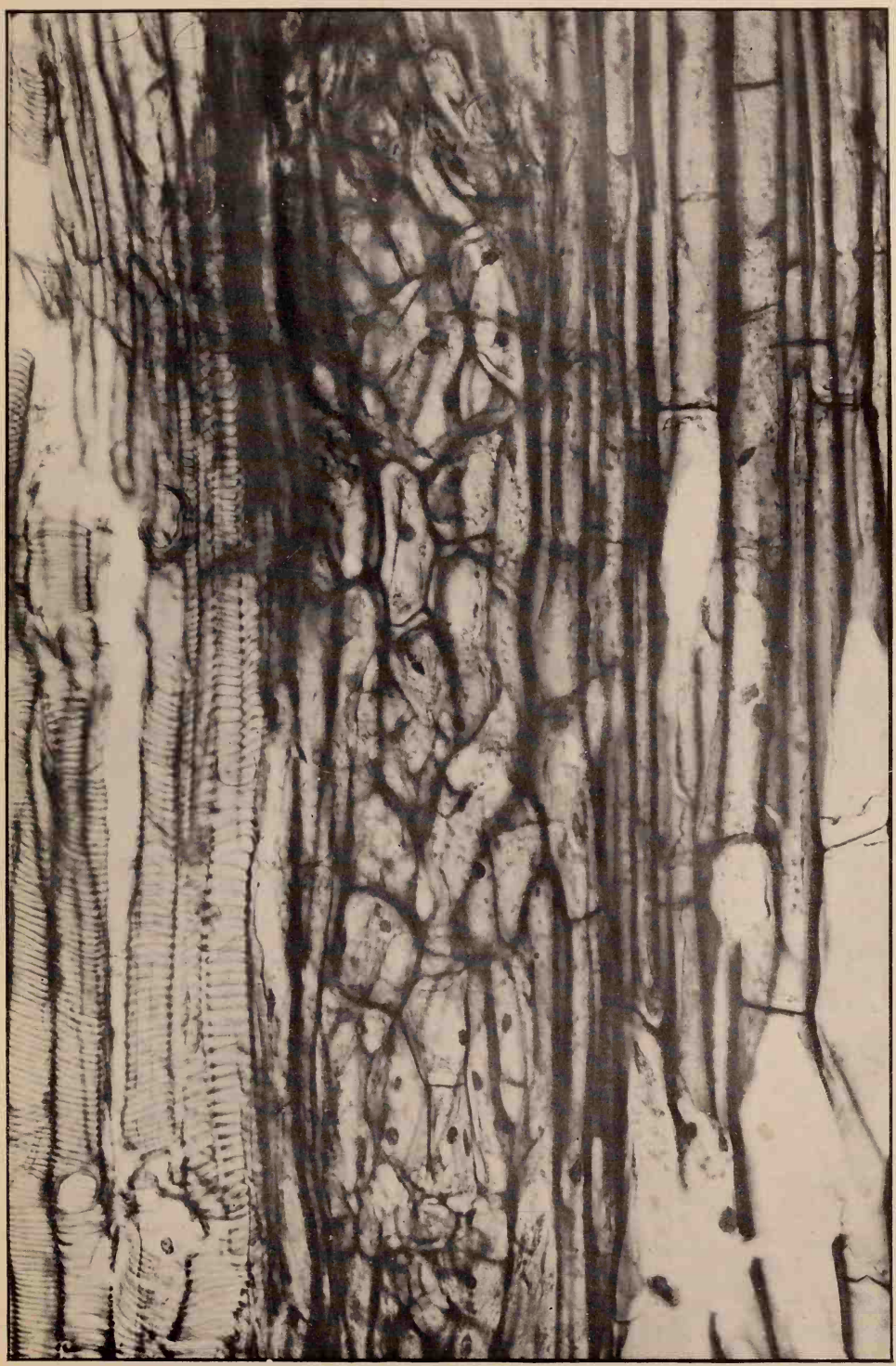
Daisy I. Longitudinal section of petiole C, showing tumor-strand (p. 28).



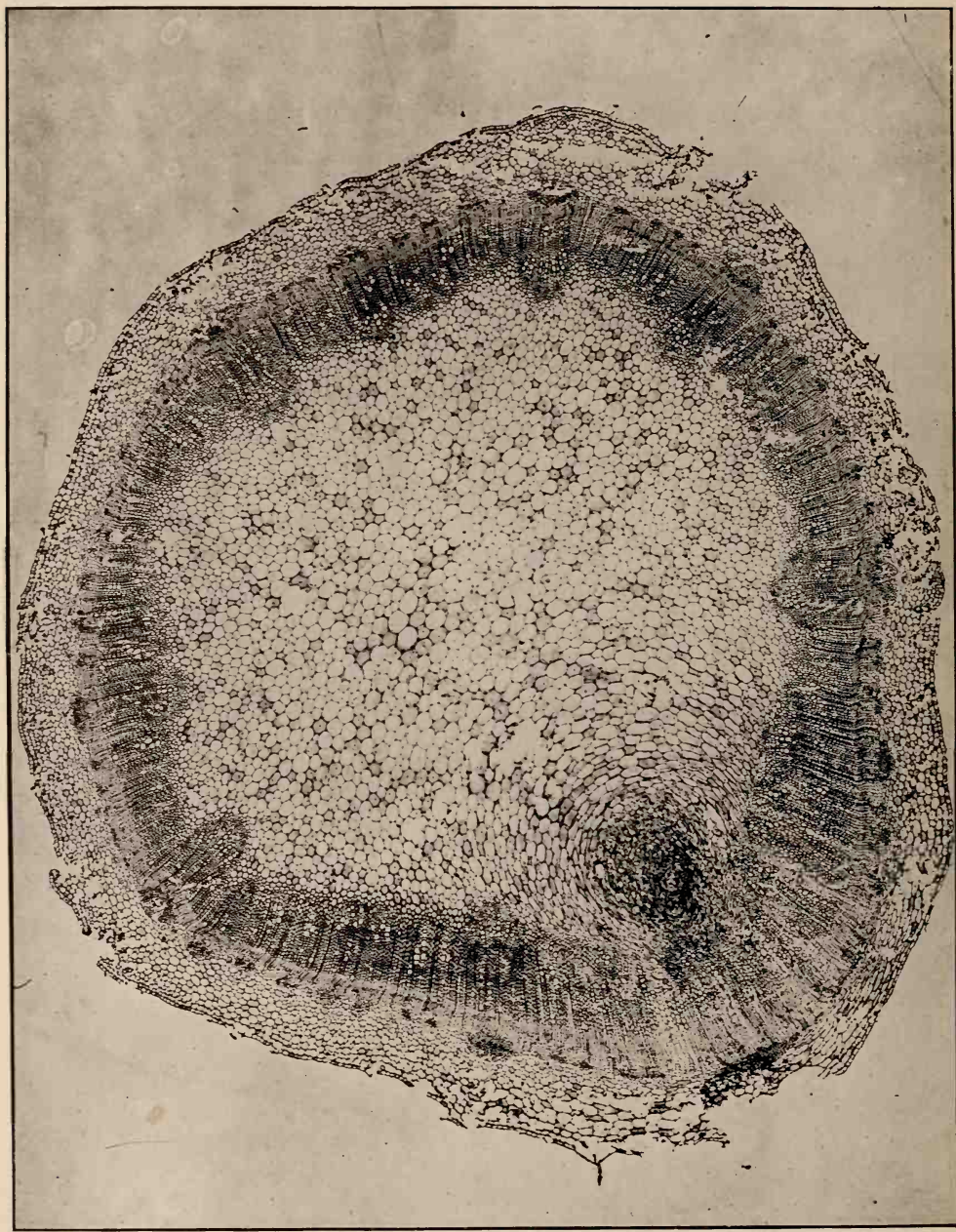
Daisy I. Continuation of Plate XVII, strand enlarging into a tumor (p. 28).



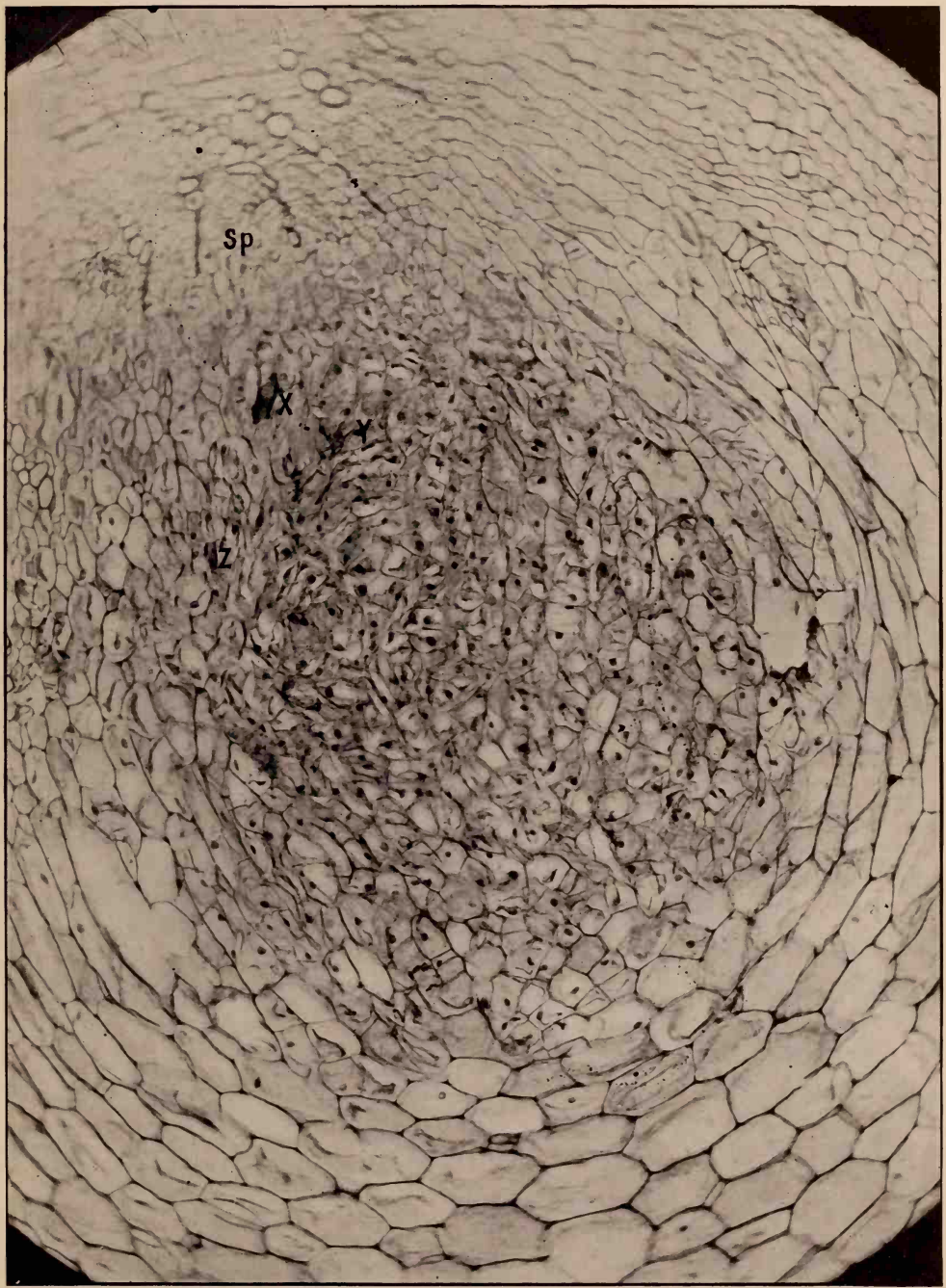
Daisy I. Continuation of Plate XVIII, showing tumor-strand (p. 28).



Daisy I. Continuation of Plate XIX, but tumor-strand further magnified (p. 29).

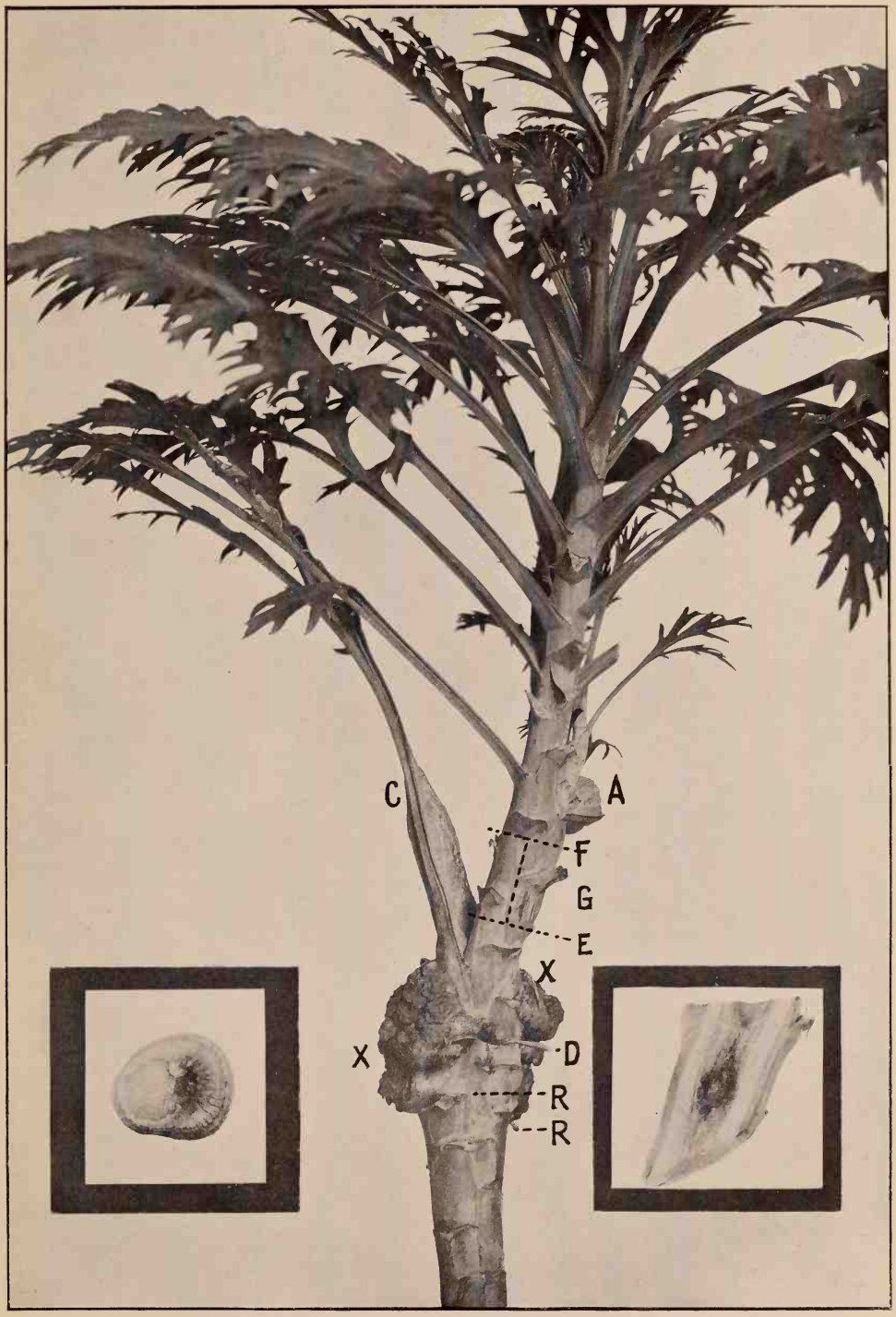


Daisy II. Cross section of stem between tumors, showing tumor-strand. The wood on that side is slightly enlarged and the pith is compressed. The rest of the stem is normal (p. 29).



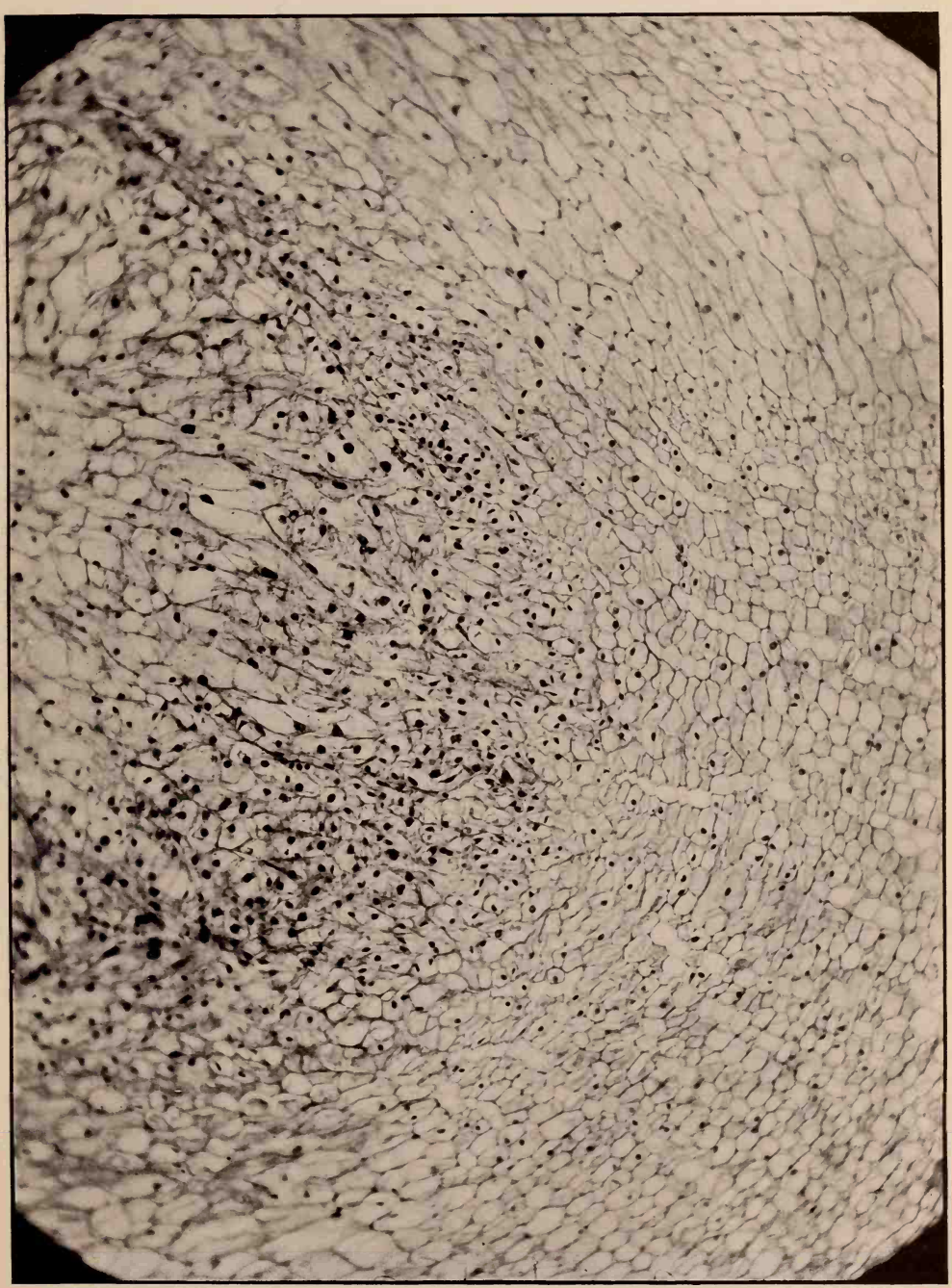
Daisy II. Same as Plate XXI, but tumor-strand further magnified; at the top crushed and displaced spirals (p. 29).

Daisy V. Inoculated plant, showing a primary stem tumor and leaves converted into secondary tumors (p. 29).

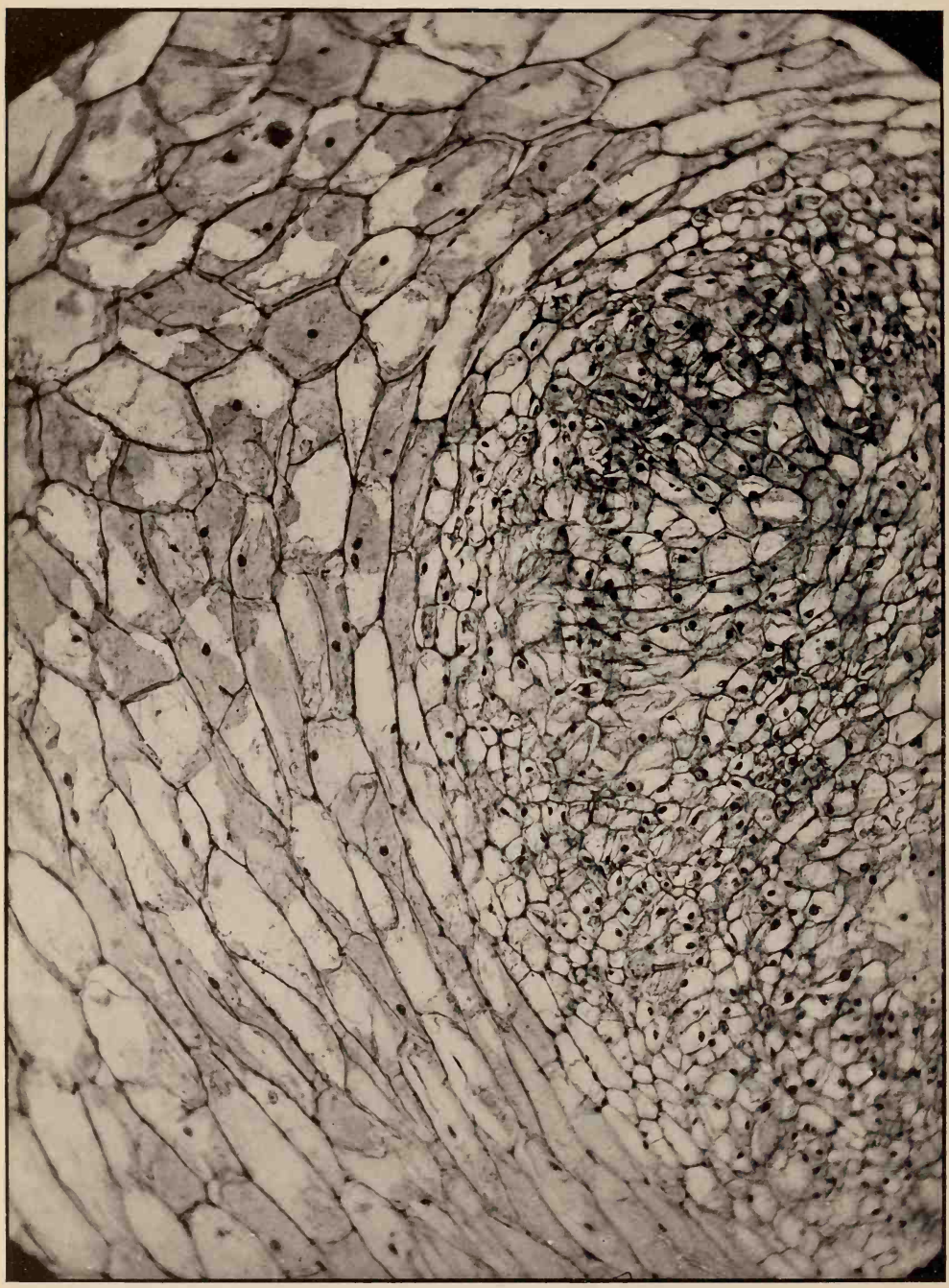


Daisy V. Other side of the plant, with sections of the abnormal stem (p. 30).

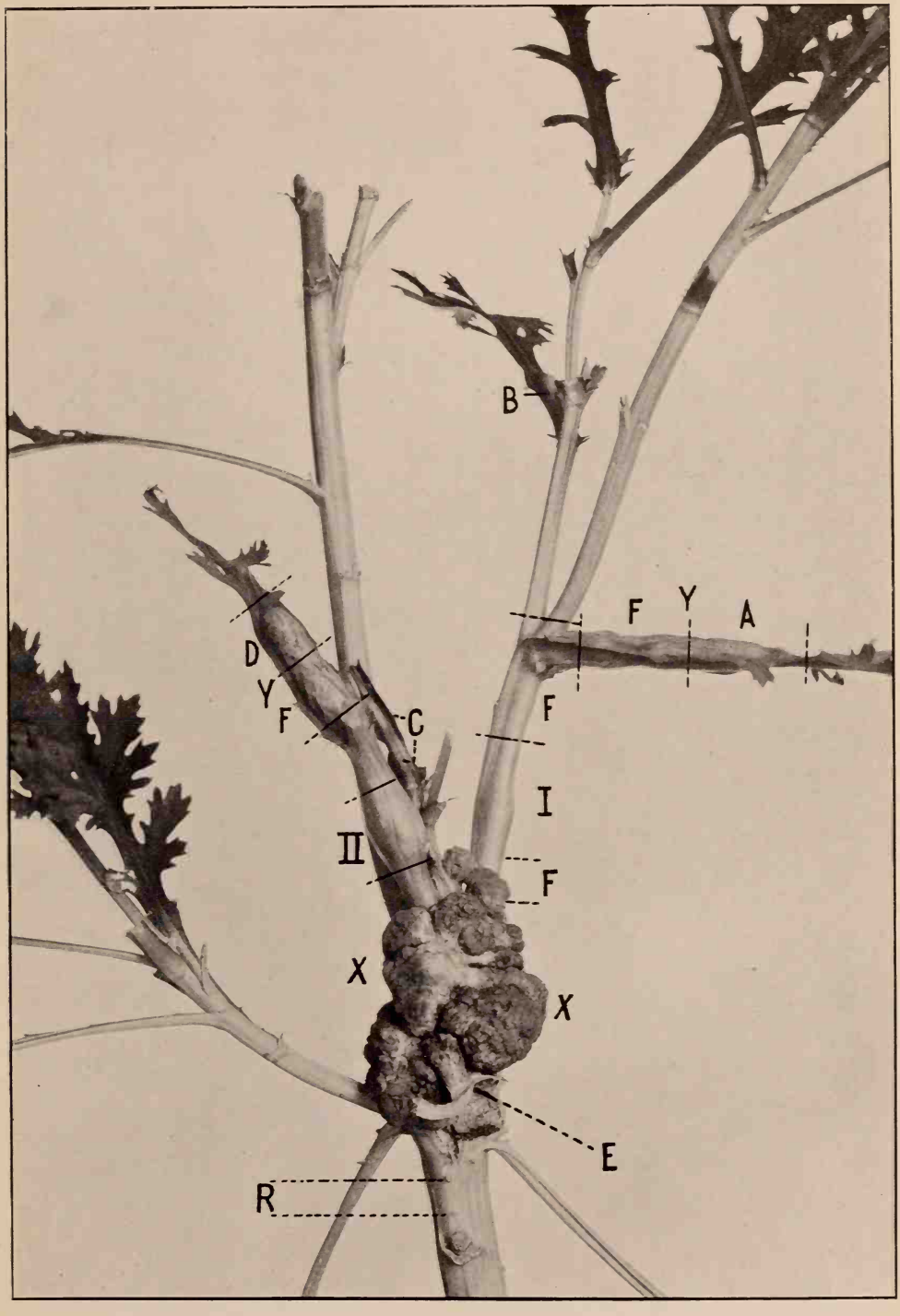
Daisy V. Enlarged cross section of stem between tumors, showing a large tumor-strand with marked enlargement of the wood on that side (p. 30).



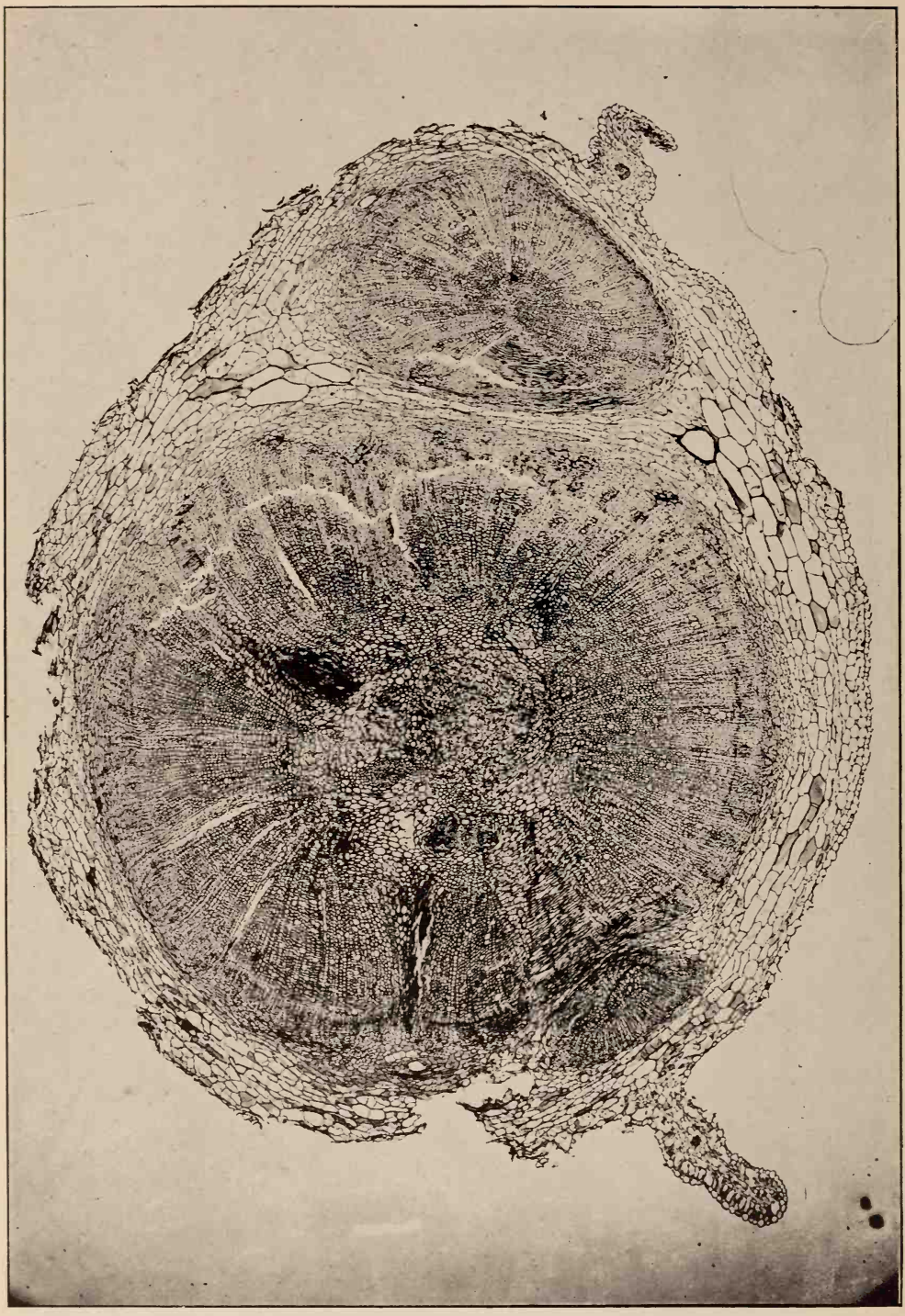
Daisy V. Detail from top of tumor-strand on Plate XXV, showing gradual transition into the wood (p. 30).



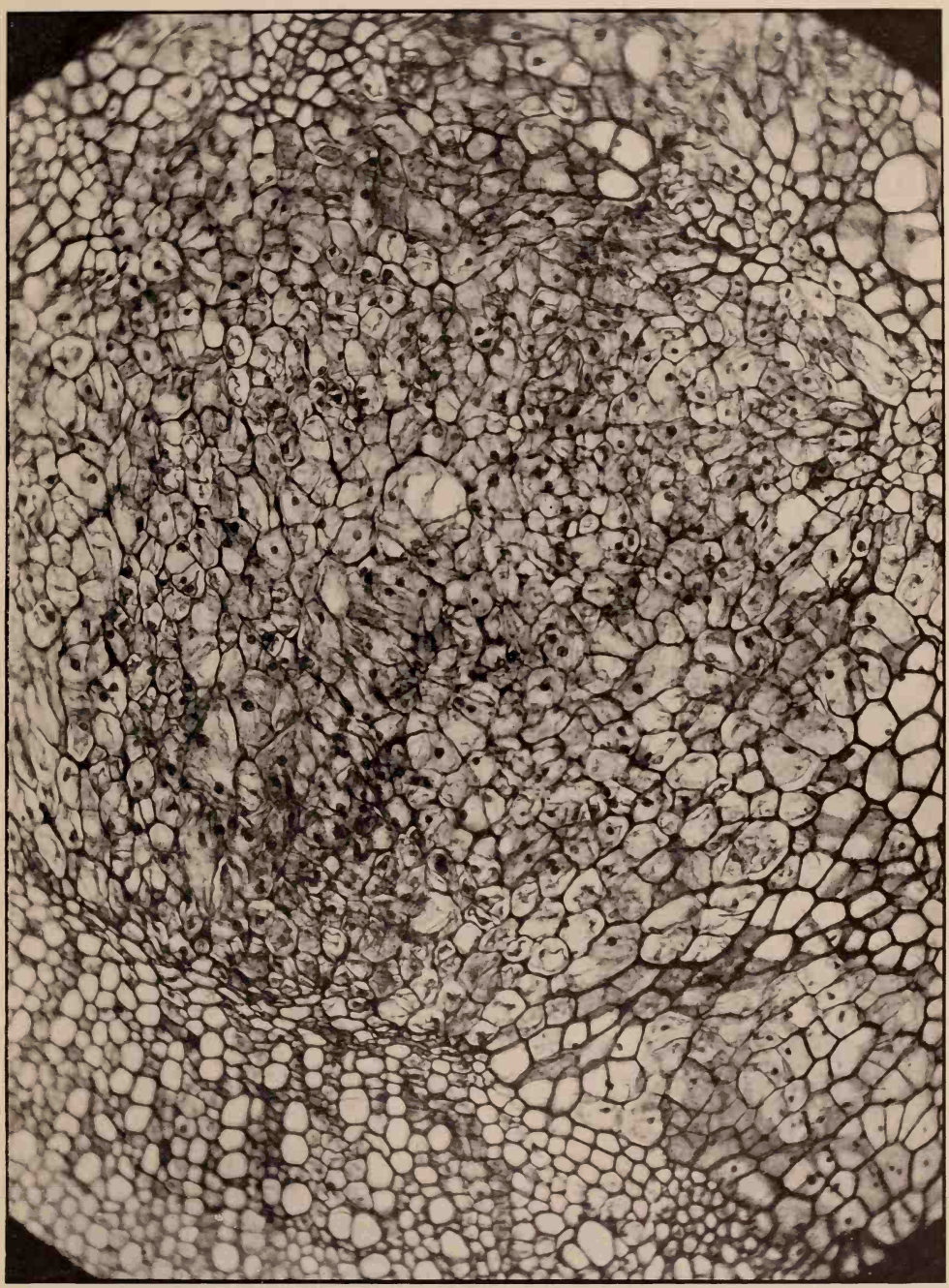
Daisy V. Detail from bottom of tumor-strand shown on Plate XXV; flattened pith at left with sharp line of demarcation (p. 30).



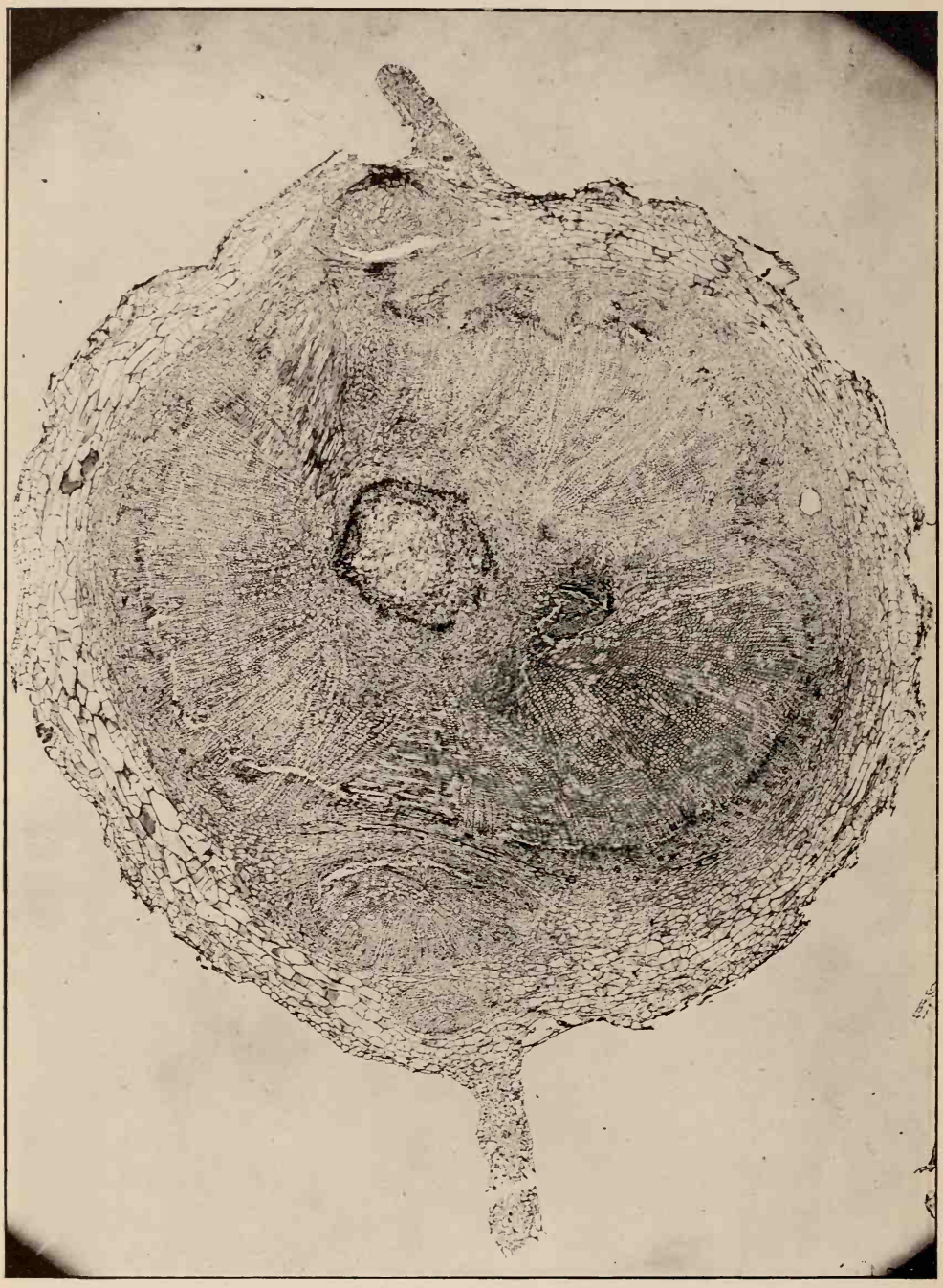
Daisy VII. Part of plant, showing primary and secondary tumors (p. 31).



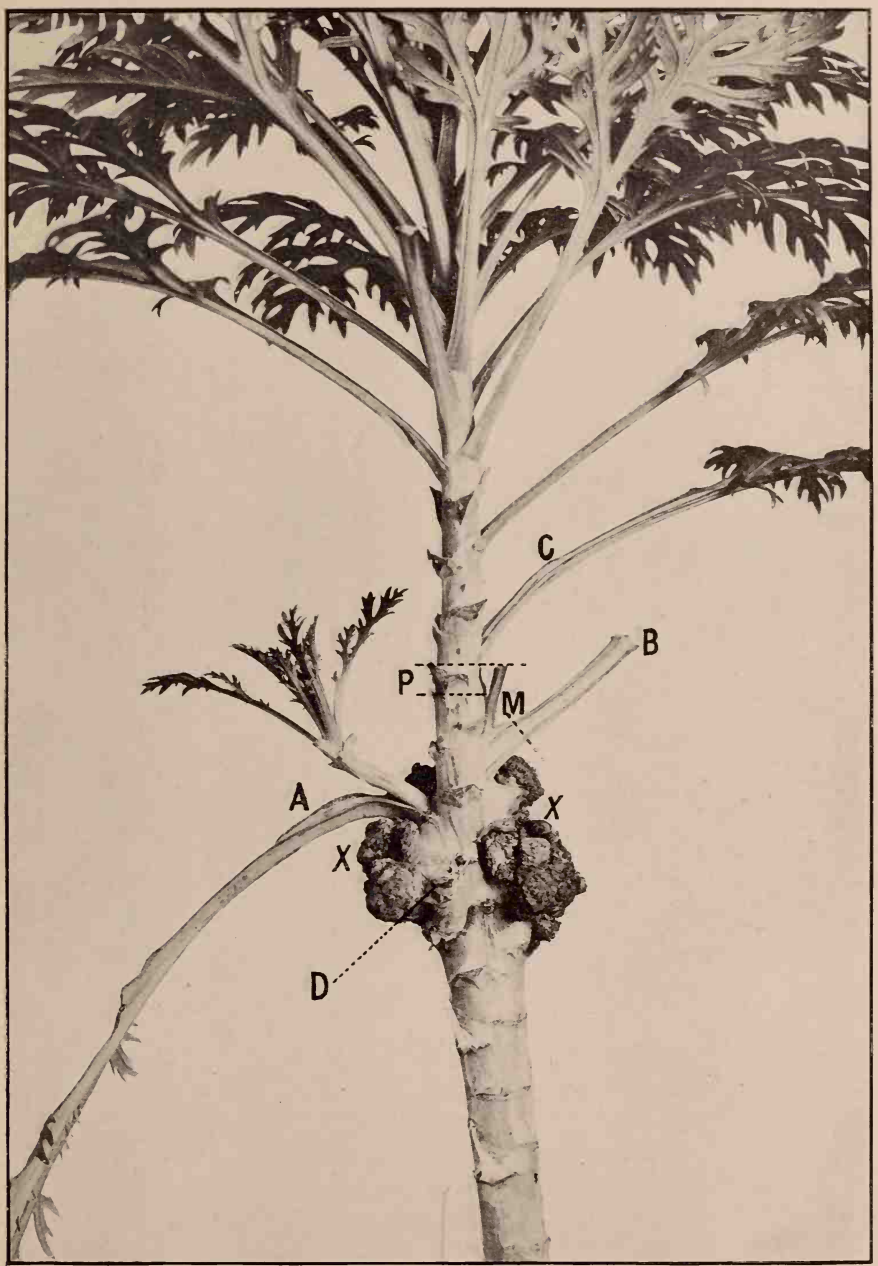
Daisy VII. Cross section of base of petiole A, showing stem structure in three leaf-traces (p. 32).



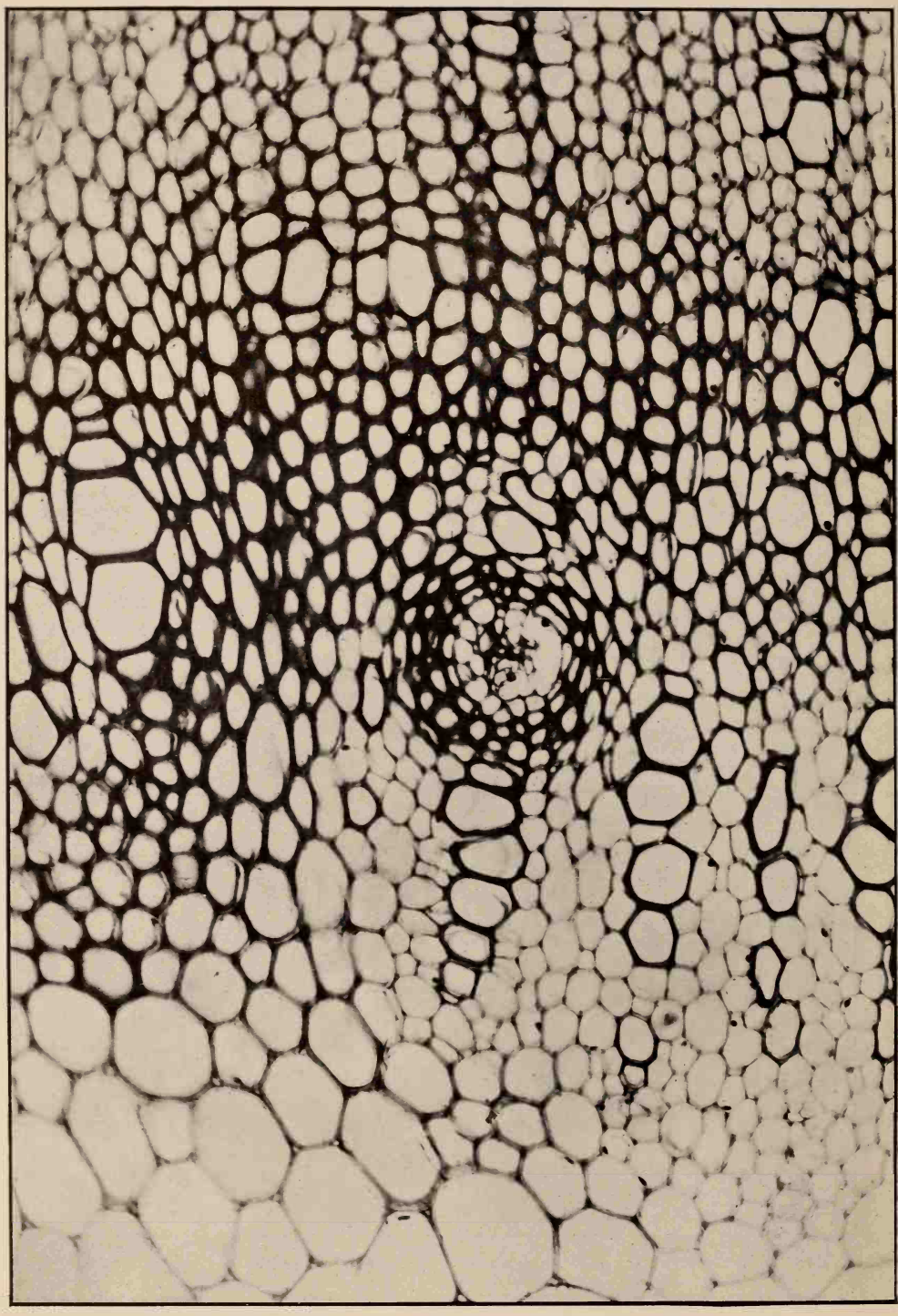
Daisy VII. Cross section of the larger tumor-strand in petiole A; abnormal medullary ray in lower right corner (p. 33).



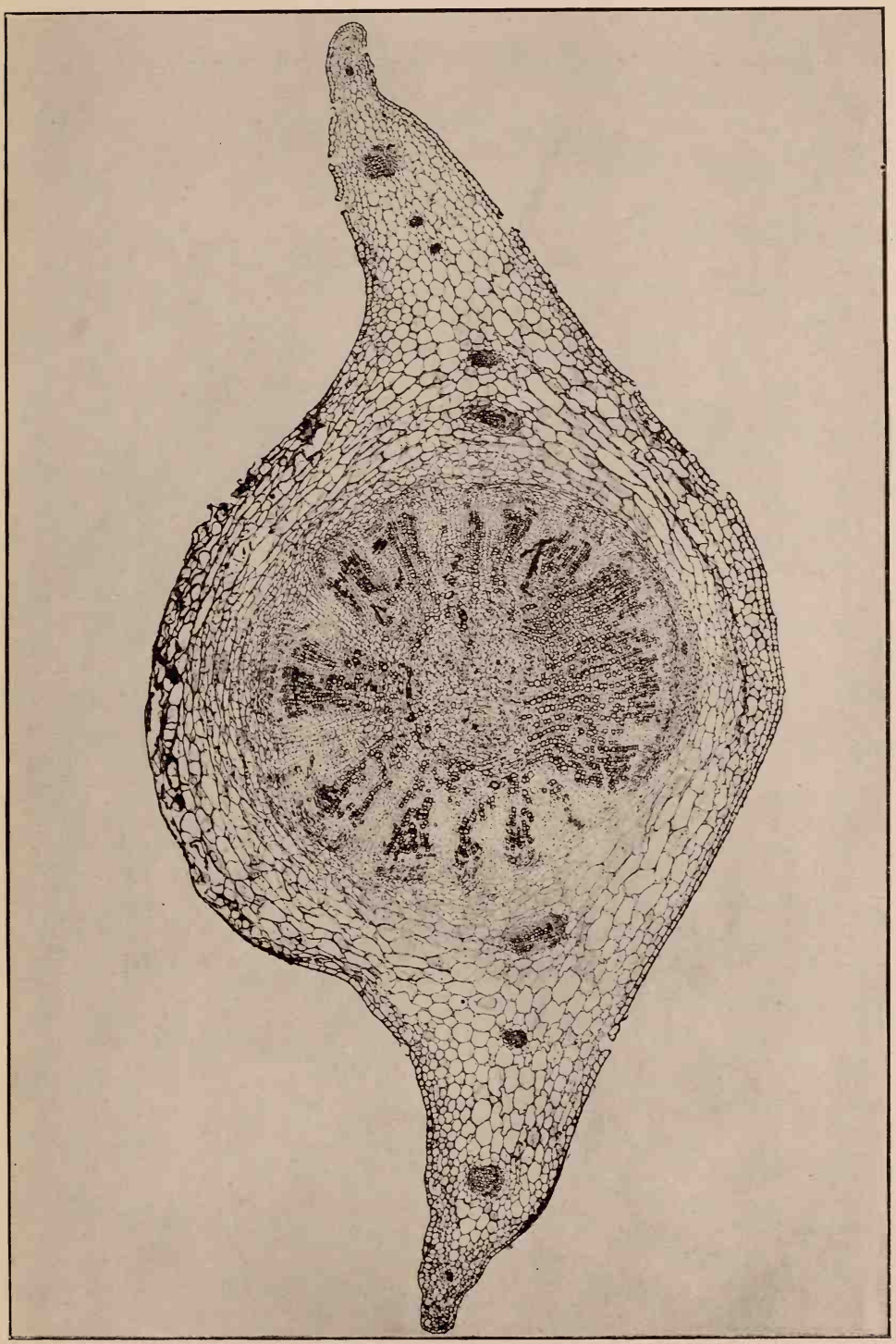
Daisy VII. Cross section of petiole D, showing stem structure (p. 33).



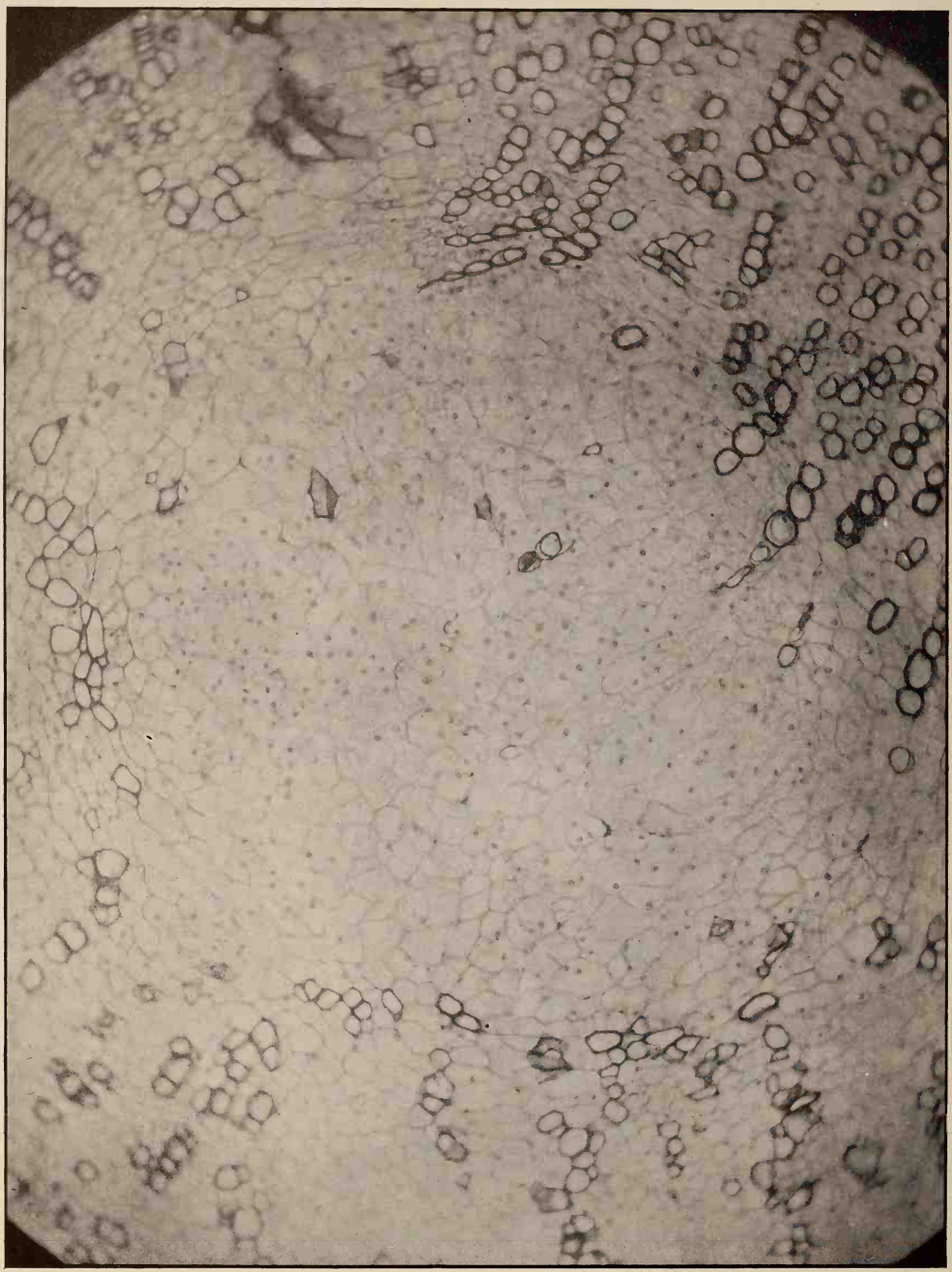
Daisy XI. Inoculated plant, showing primary stem tumor and secondary leaf tumors (p. 33).



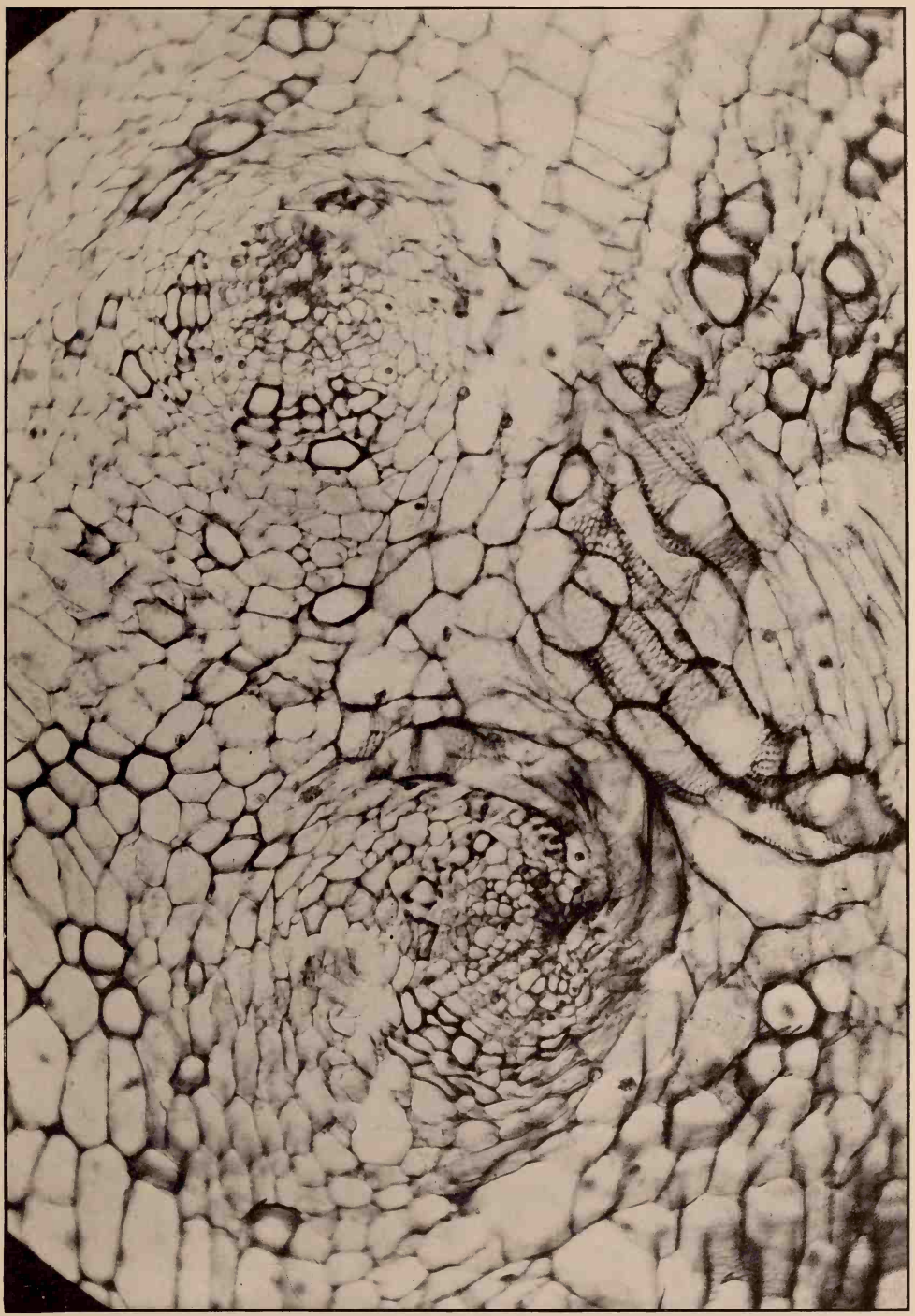
Daisy XI. Cross section of stem in vicinity of P, showing strand (p. 34).



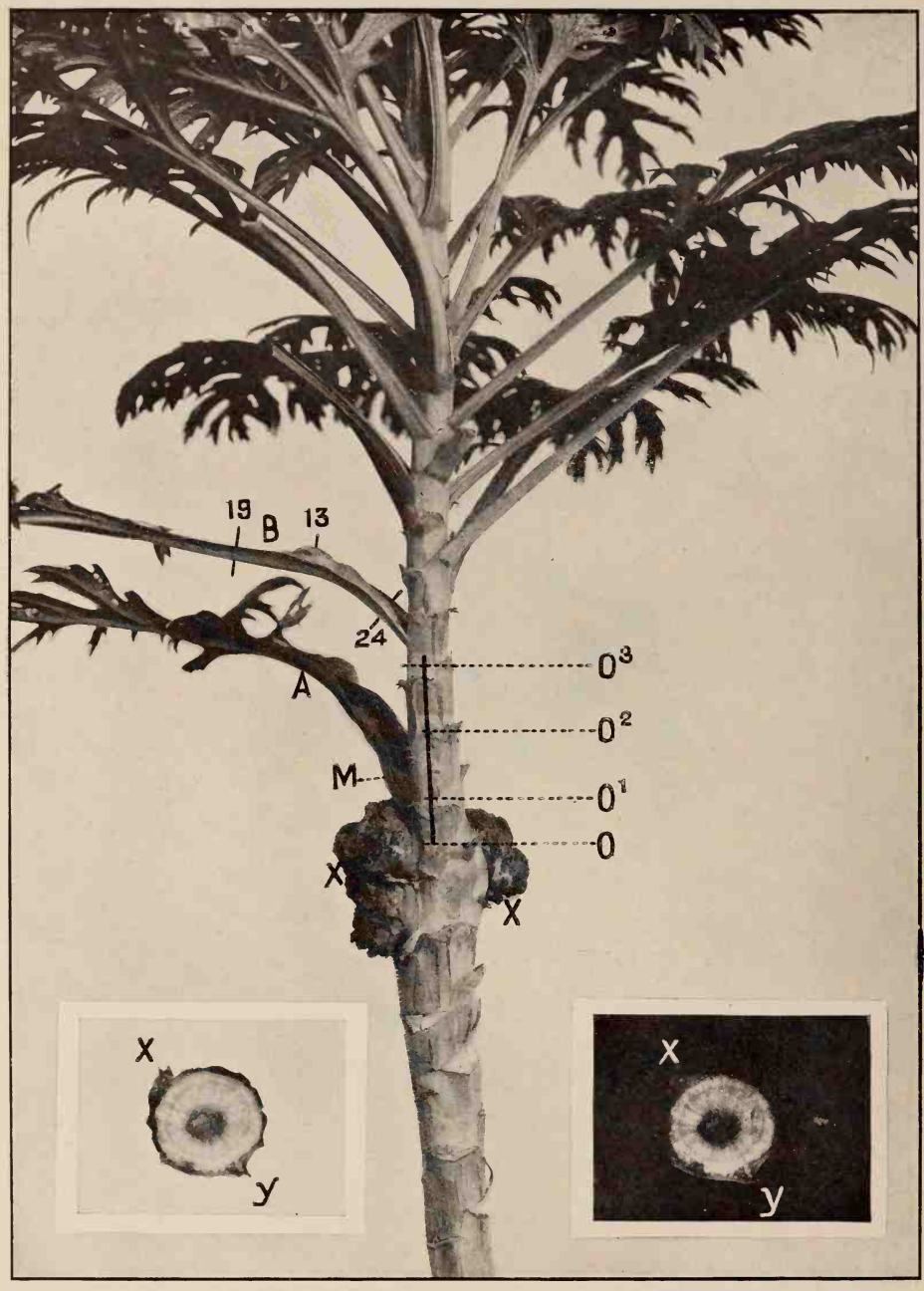
Daisy XI. Cross section of petiole B at M (Pl. XXXII), showing stem tumor in center (p. 34).



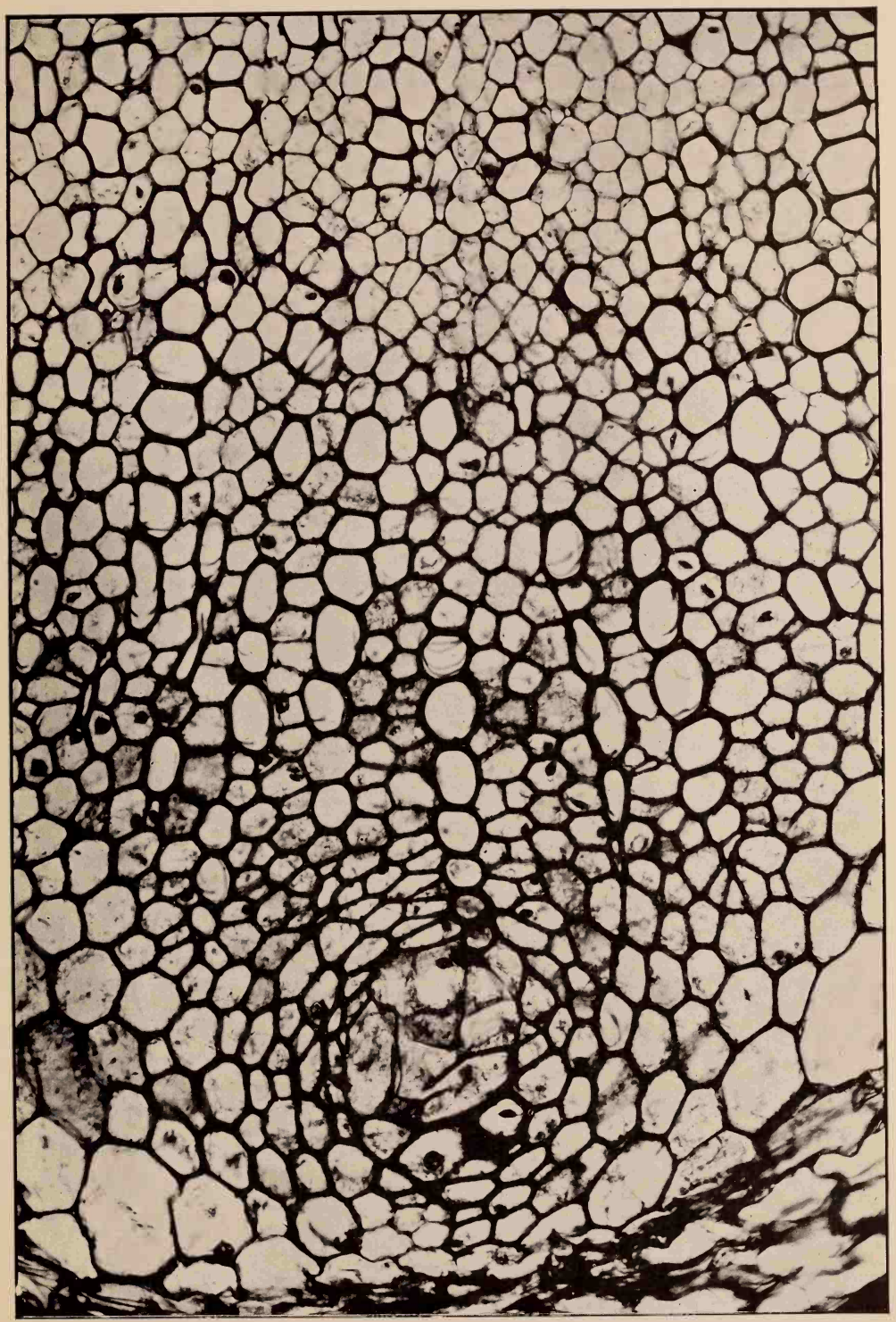
Daisy XI. Cross section of tumor-strand in center of Plate XXXIV, showing tracheids therein and torn and crushed spirals (p. 34).



Daisy XI. Whorls of tumor tissue and twisted tracheids in petiole B (p. 34).



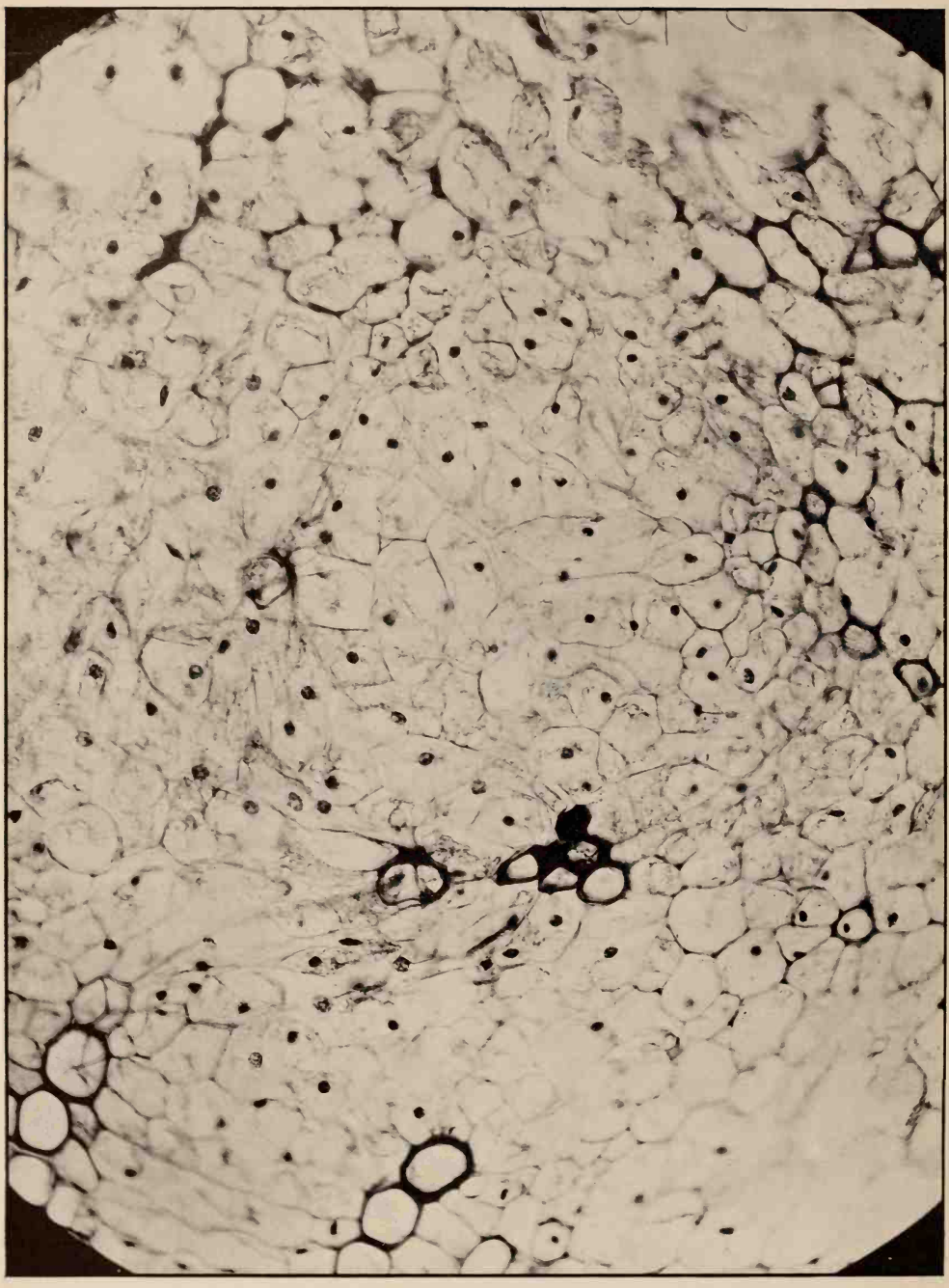
Daisy XII. Primary tumor and secondary tumors; also enlarged cross section of secondary tumor in a leaf (p. 35).



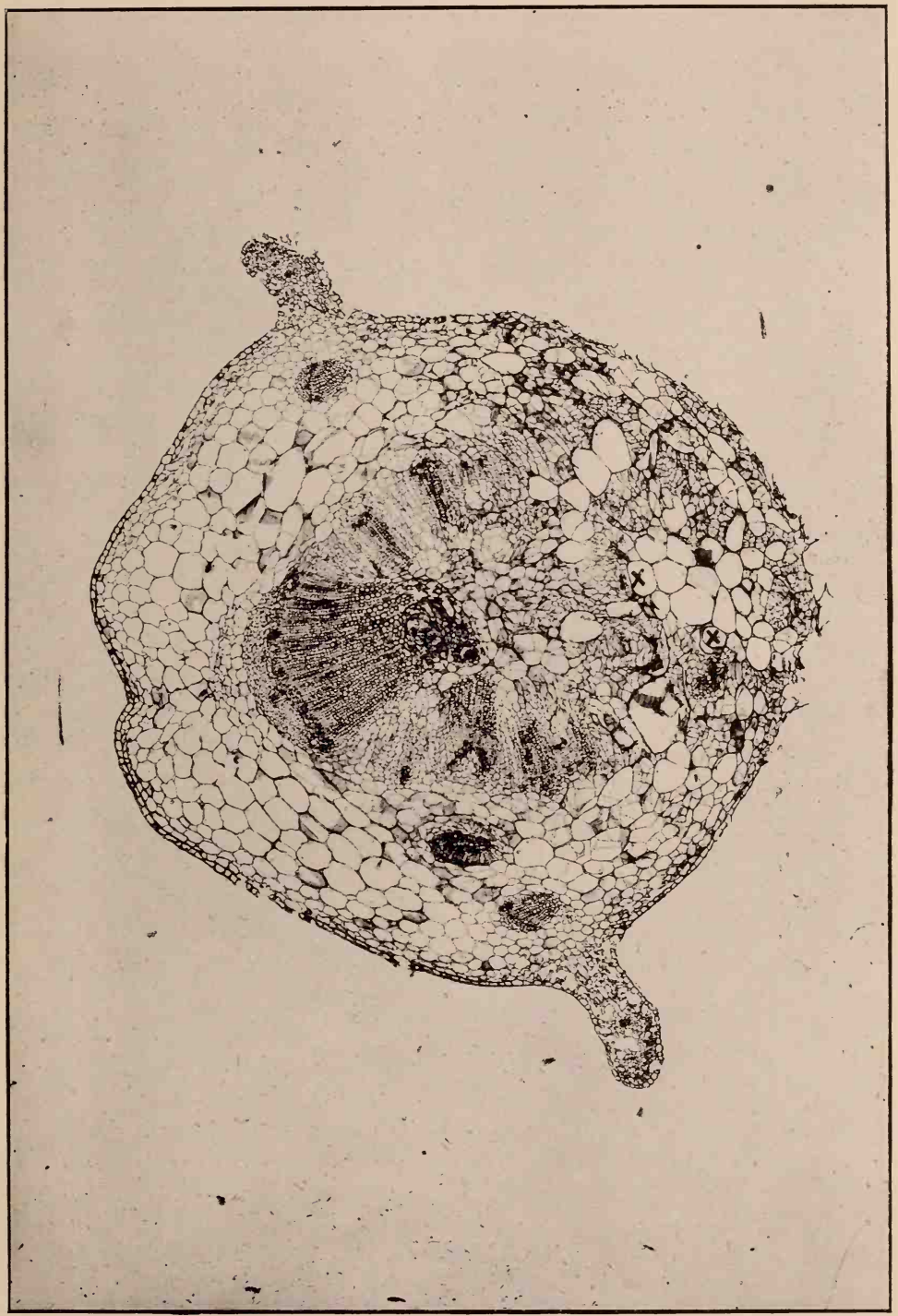
Daisy XII. Cross section of stem between tumors, showing tumor-strand (p. 35).

Daisy XII. Cross section of petiole A; whorl in tumor tissue (p. 36).

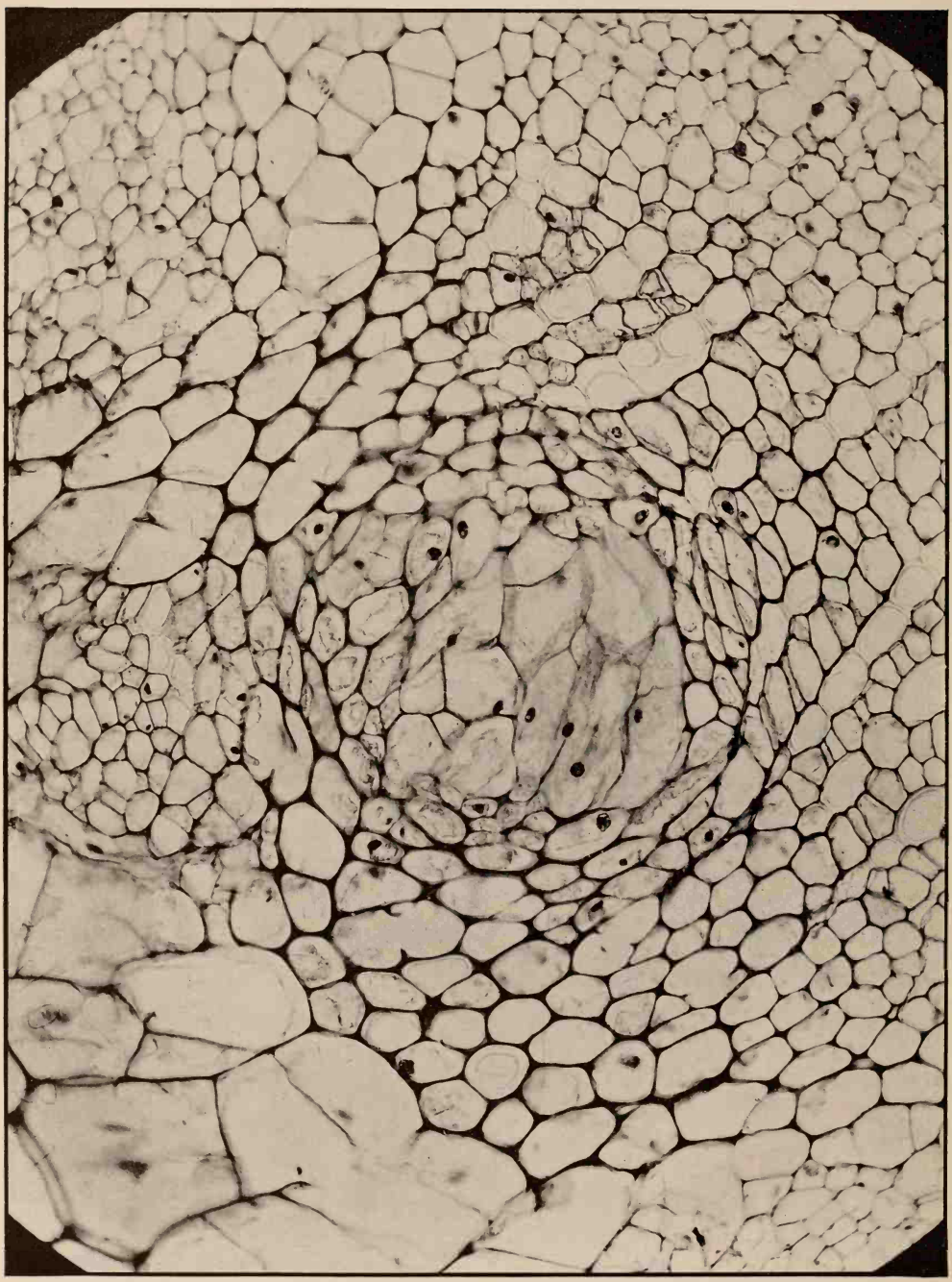
Daisy XII. Joins onto Plate XXXIX; another whorl in the tumor, X joins X (p. 36).



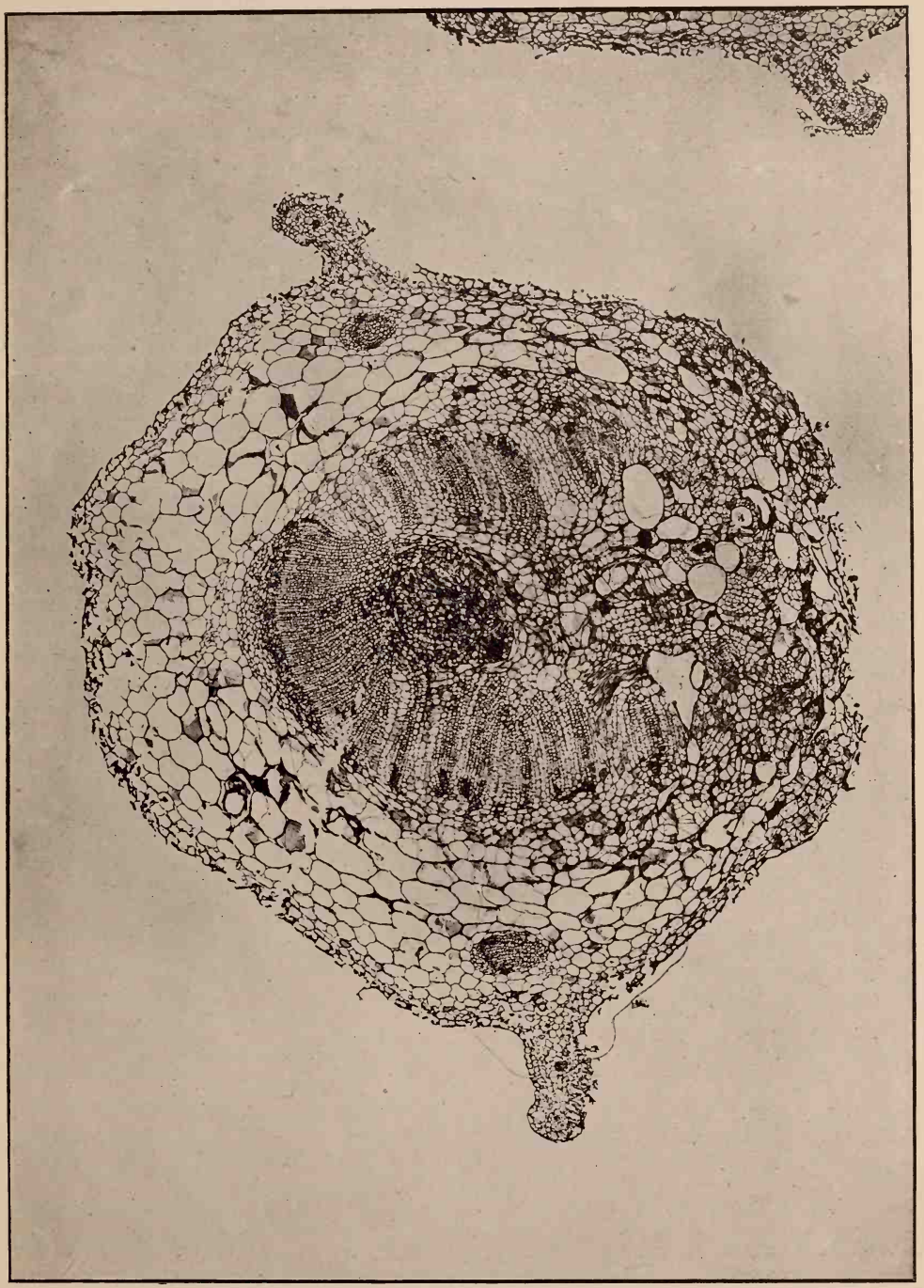
Daisy XII. Cross section of tumor-strand in petiole A, showing displaced vessels in the strand (p. 36).



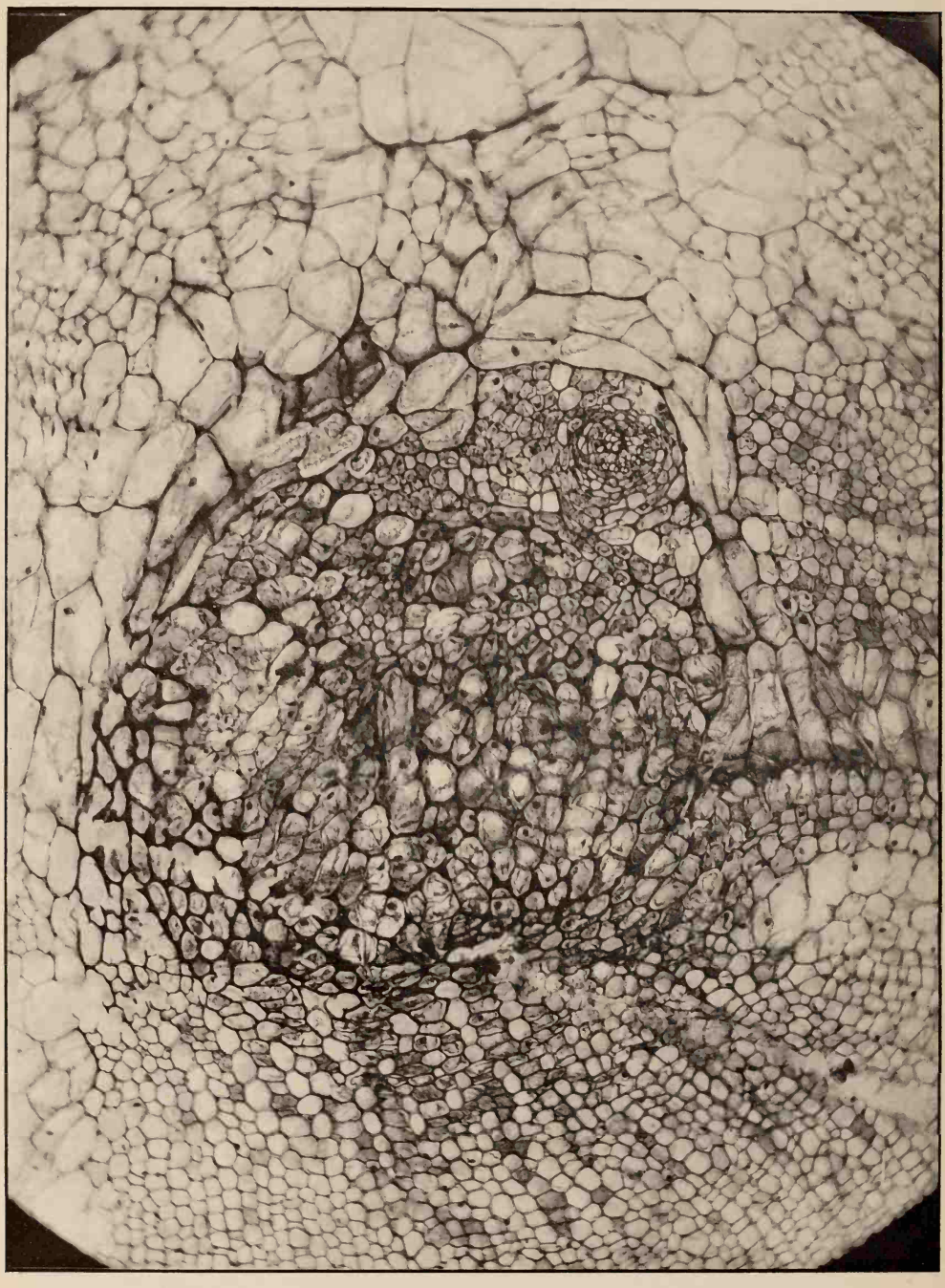
Daisy XII. Cross section of petiole B, showing a growing secondary tumor with cell inclusions (p. 36).



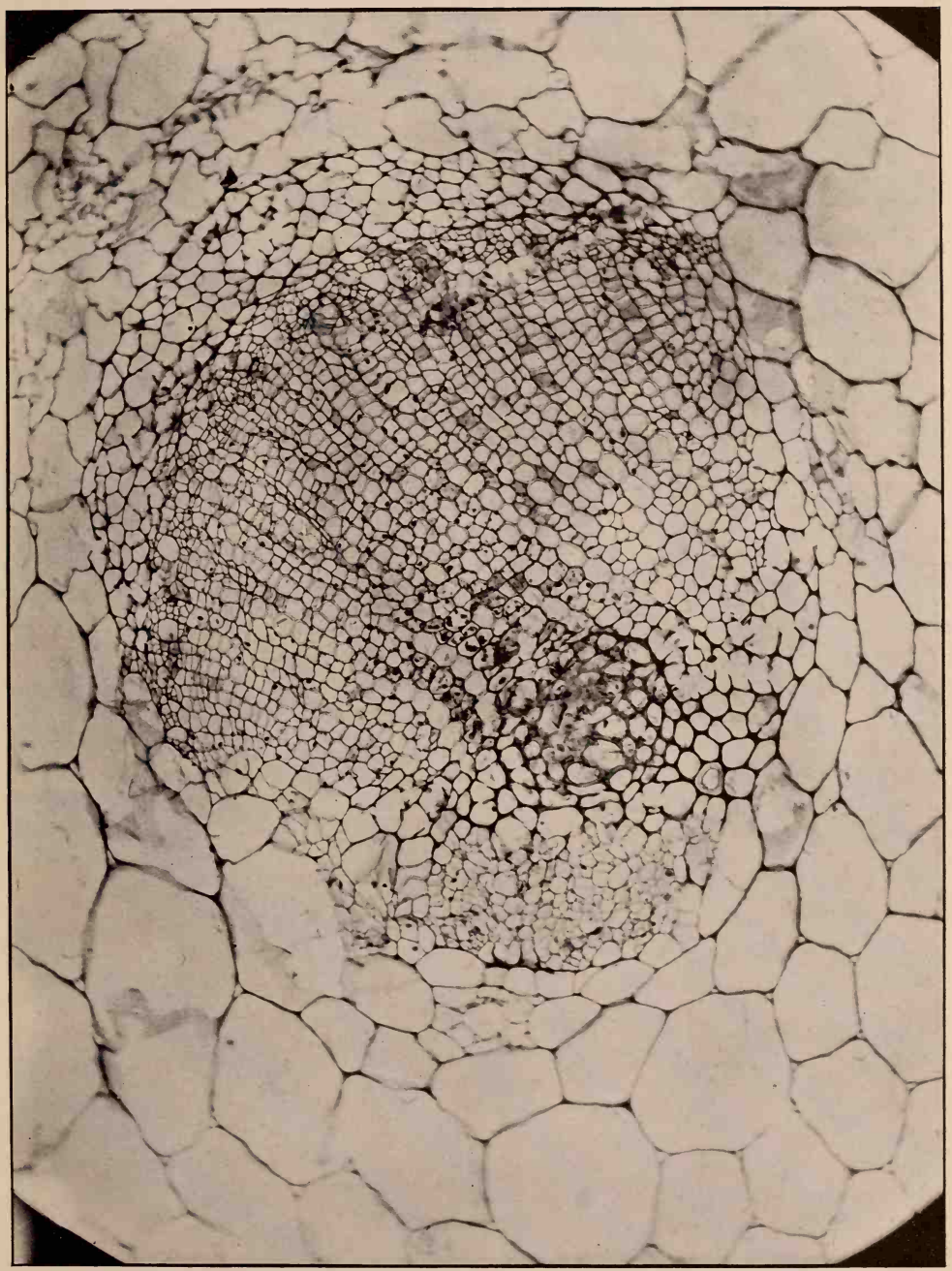
Daisy XII. Cross section, tumor-strand in petiole B, near XLII (p. 37).



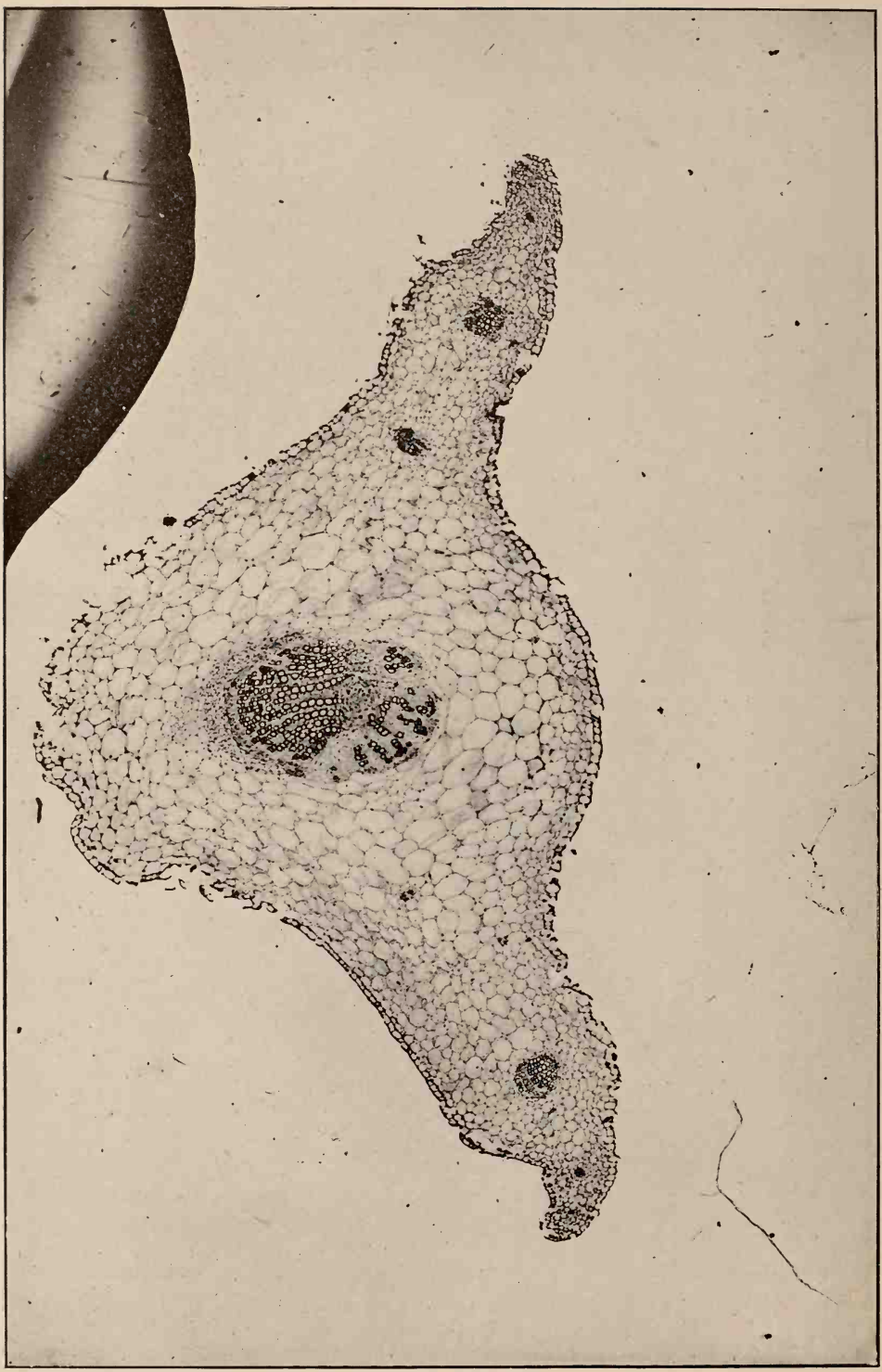
Daisy XII. Cross section of petiole B at another level, showing cell inclusions and large tumor-strand, with normal part of leaf-trace at left (p. 37).



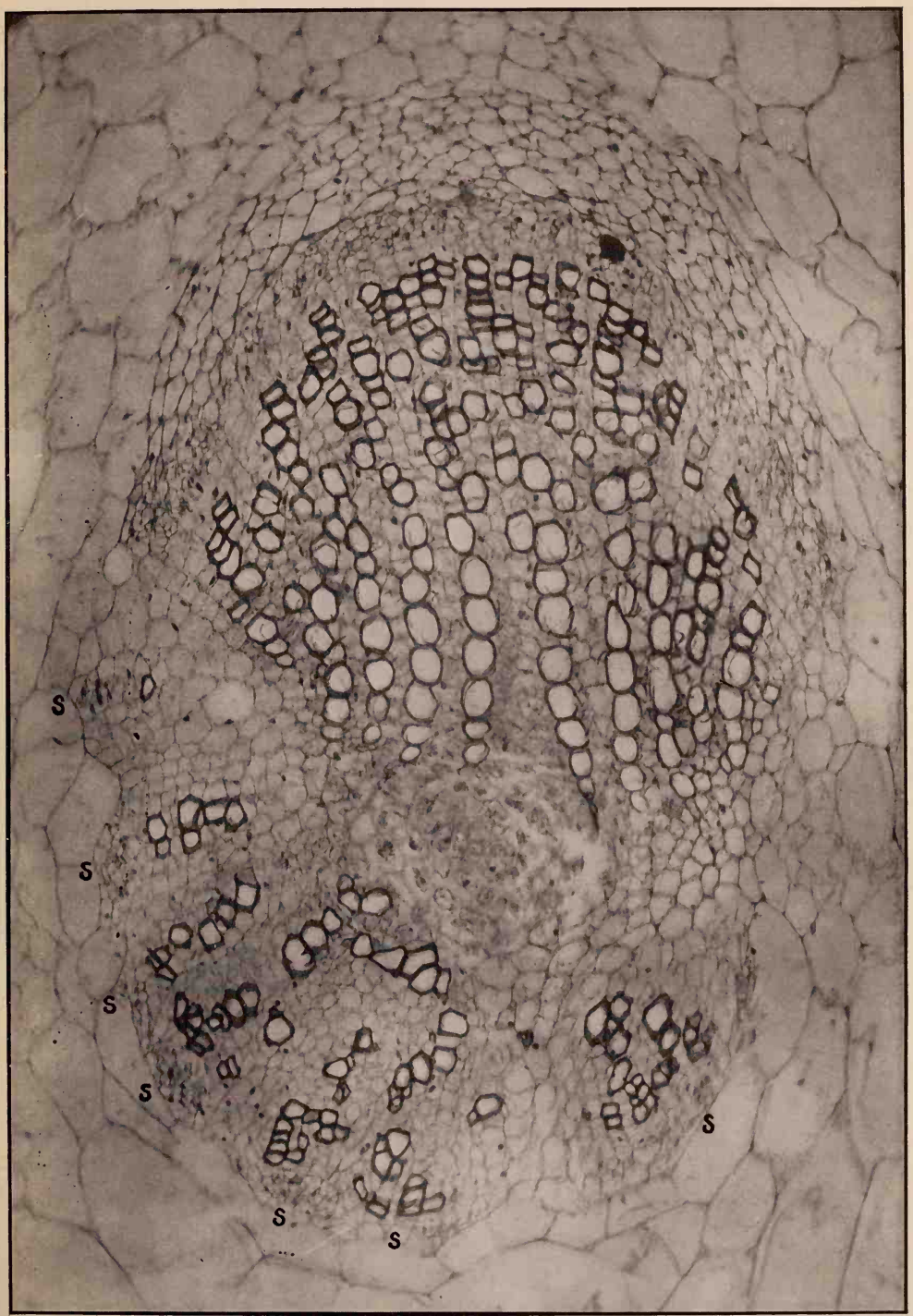
Daisy XII. Tumor-strand from Plate XLIV; large and small cells present; abnormal medullary ray at right (p. 37).



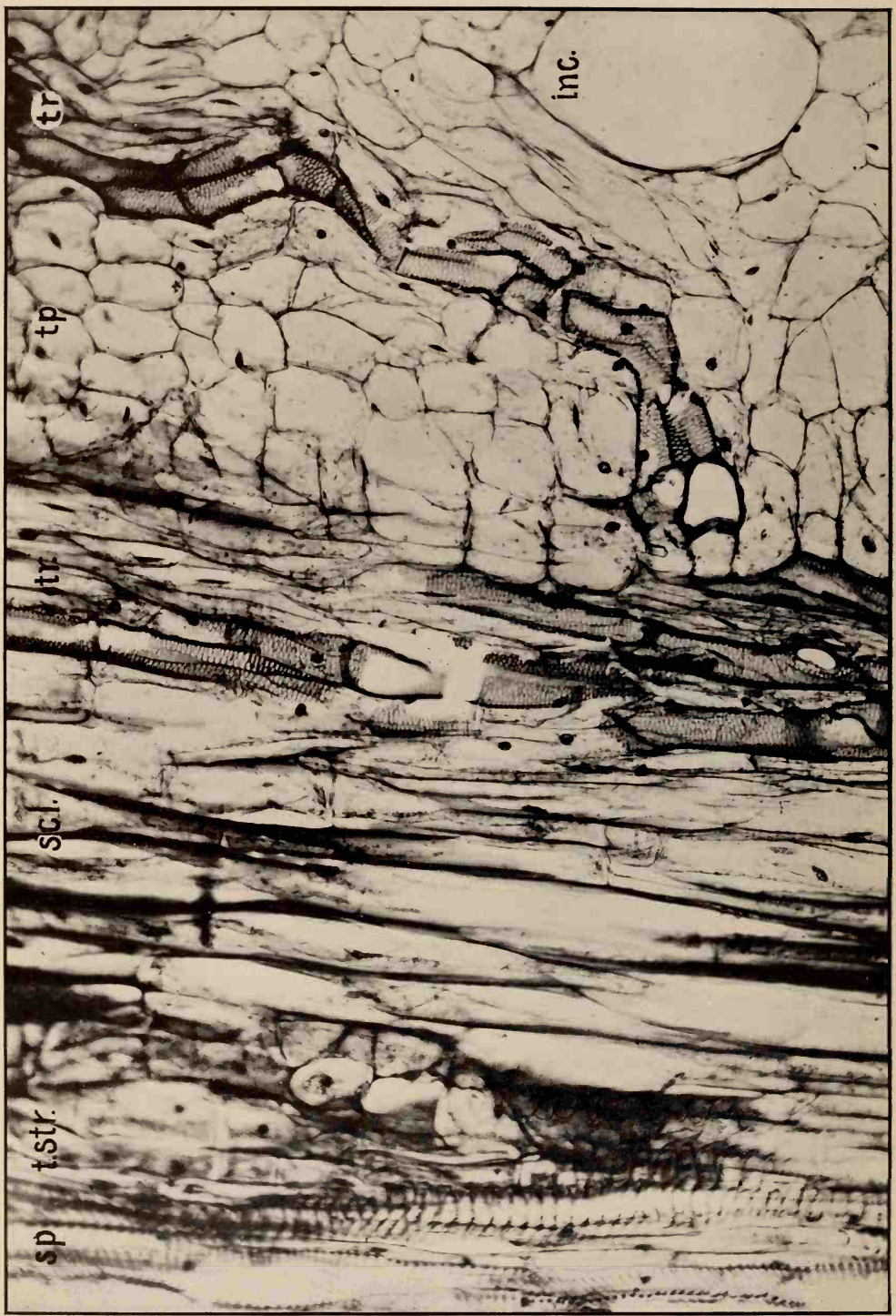
Daisy XII. Infected leaf-trace far out on petiole B (p. 37).



Daisy XII. Cross section of lower part of petiole B, showing incipient tumor in the central leaf-trace (p. 38).

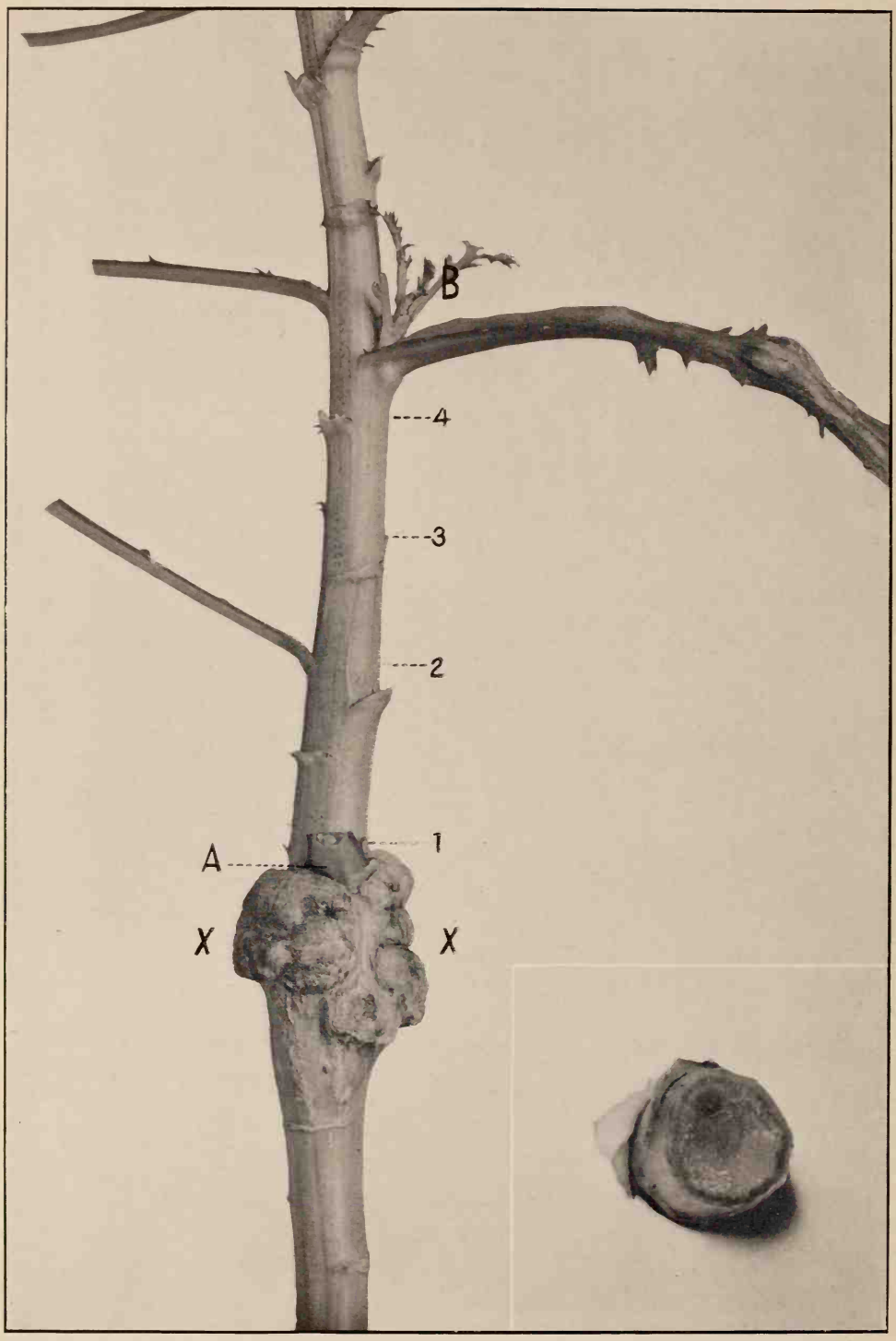


Daisy XII. Cross section of central leaf-trace of petiole B, further enlarged for comparison with Plate III (p. 38).

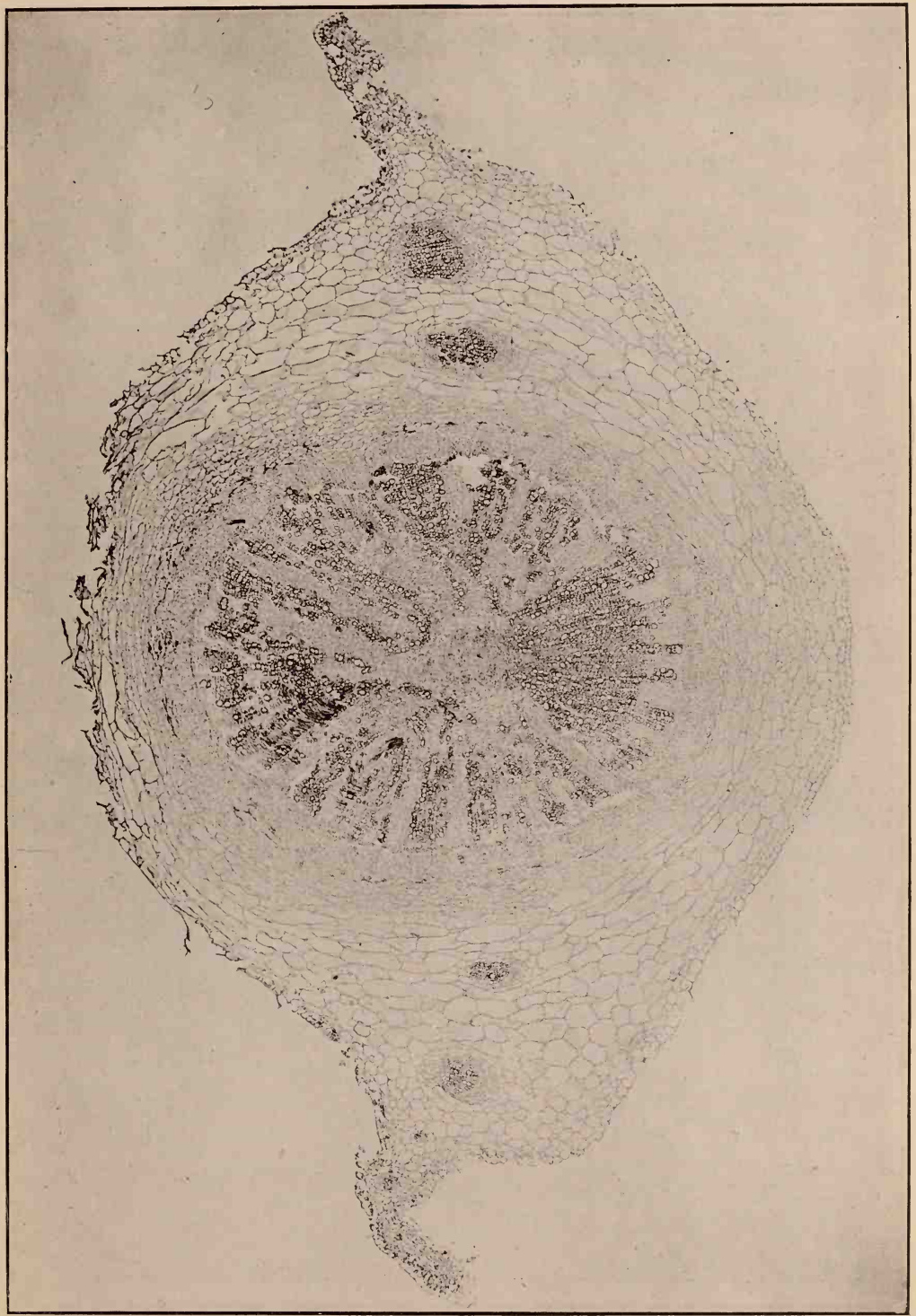


Daisy XII. Longitudinal section of leaf-trace shown in Plate XLVIII, all abnormal except sp and scl (p. 38).

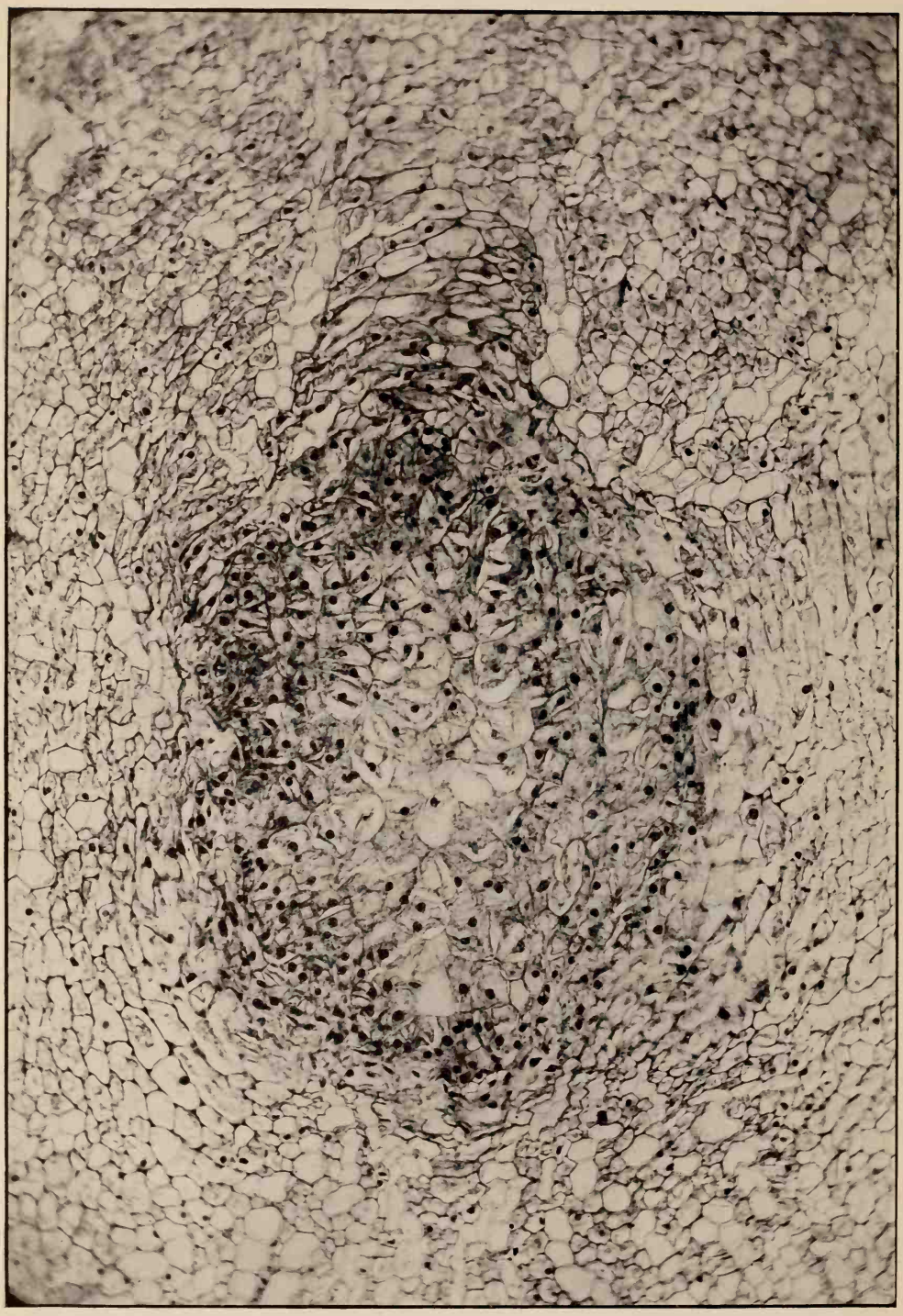
Daisy XII. Cross section showing abnormal lignification of cell-walls in the tumor (p. 38).



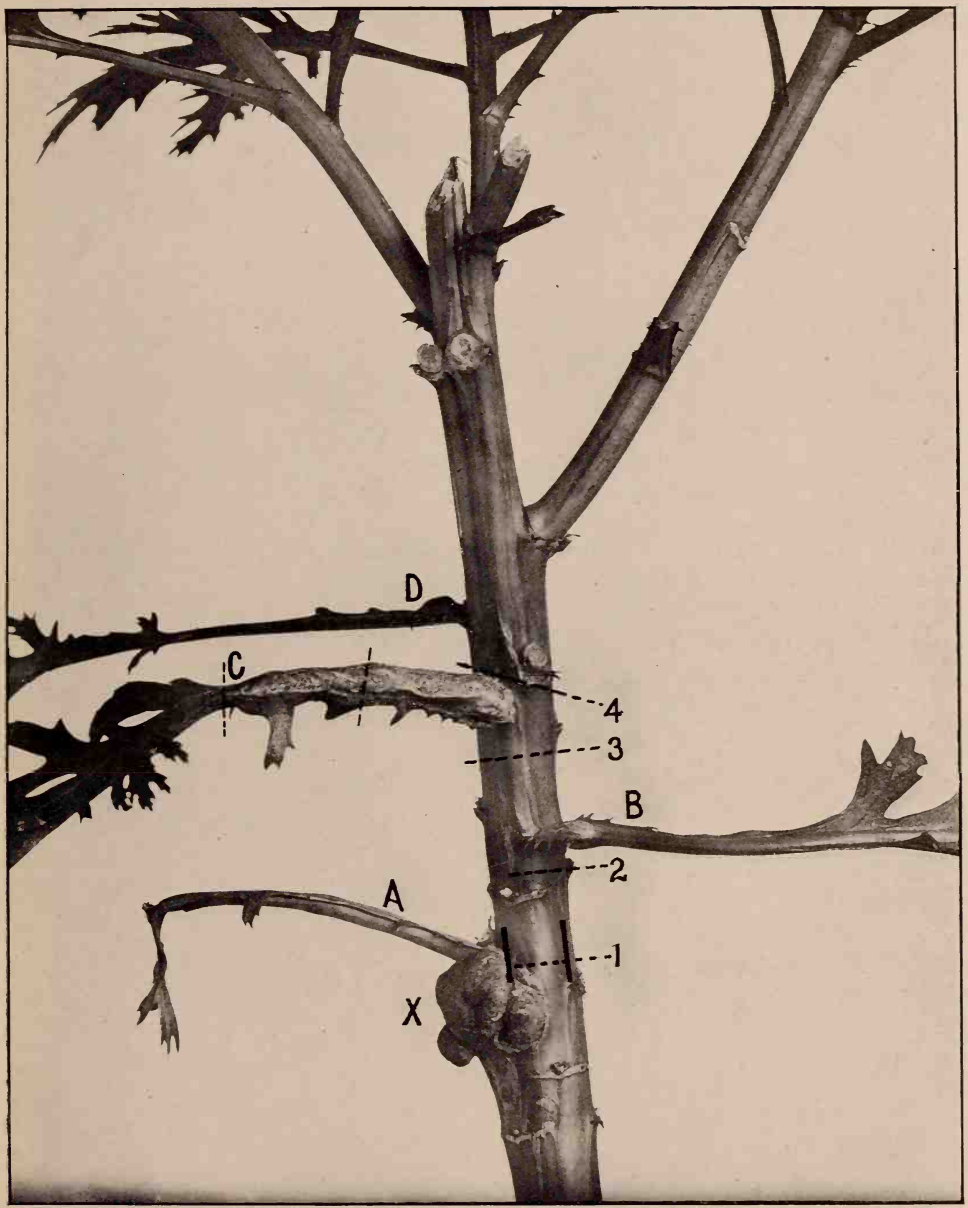
Daisy XIII. Inoculated plant, showing primary stem tumor and secondary leaf tumors; also section of stem at fig. 2 (p. 39).



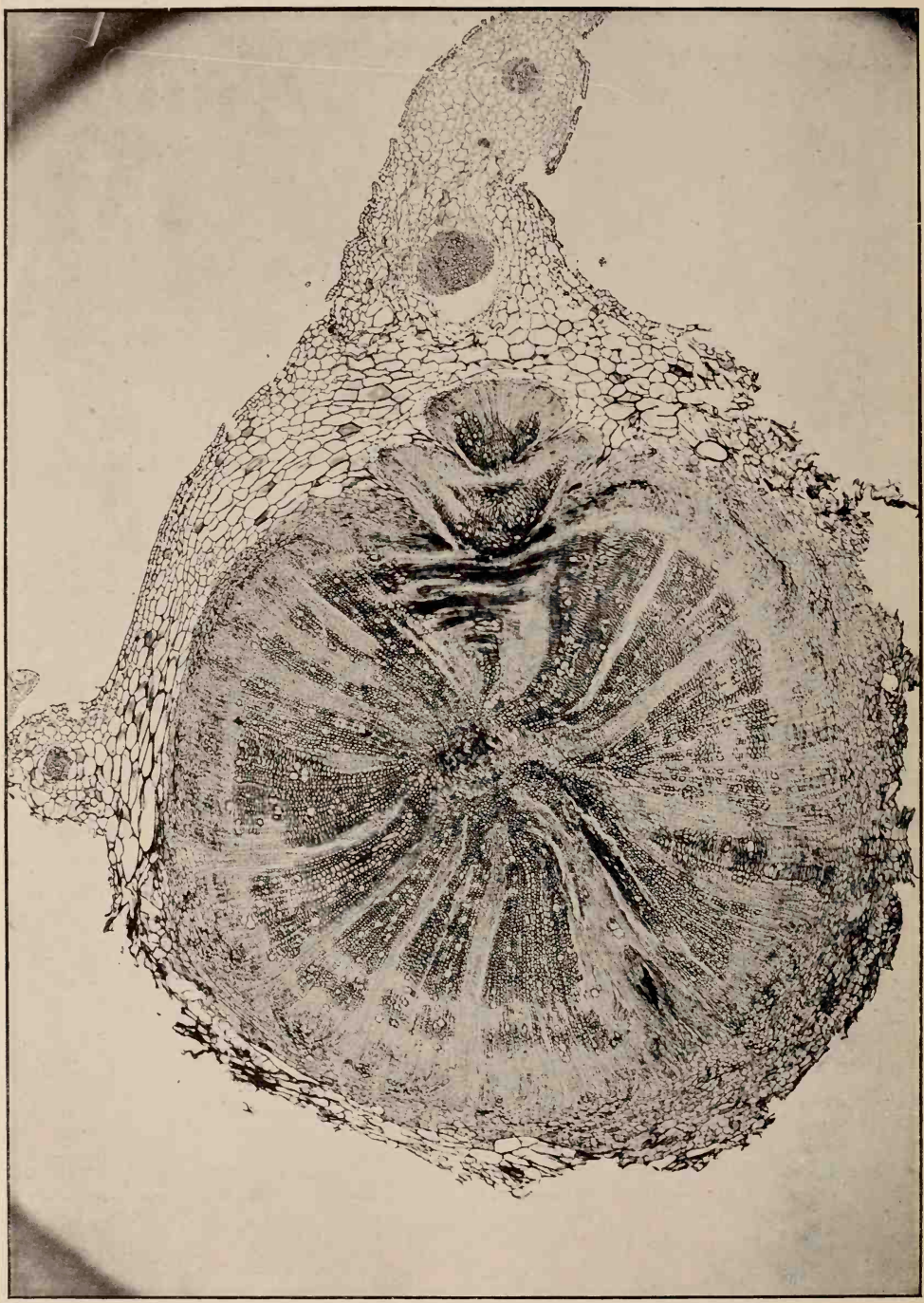
Daisy XIII. Cross section of petiole B, showing young secondary tumor in leaf-trace (p. 39).



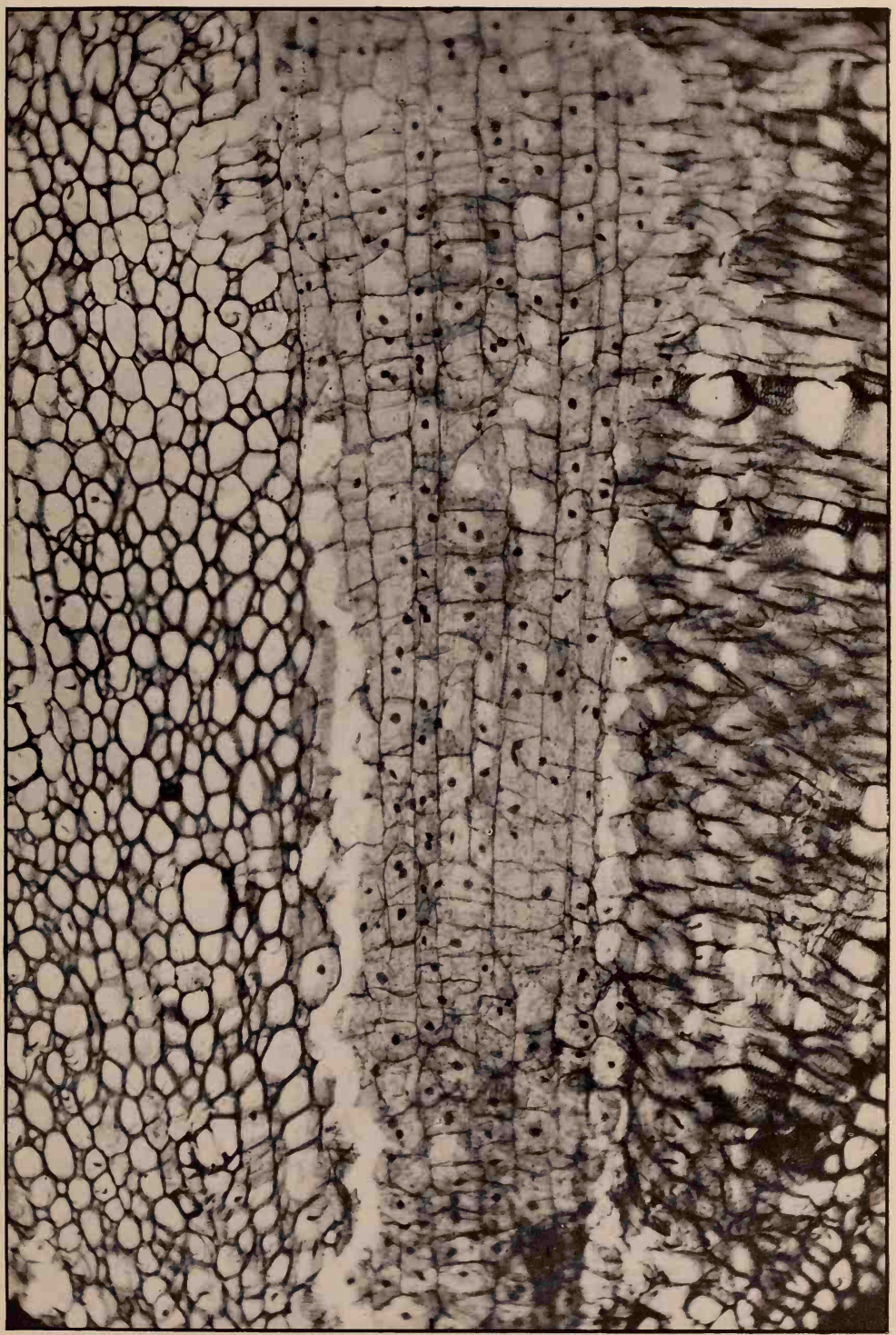
Daisy XIII. Tumor-strand enlarged from Plate LII, nuclei deeply stained (p. 40).



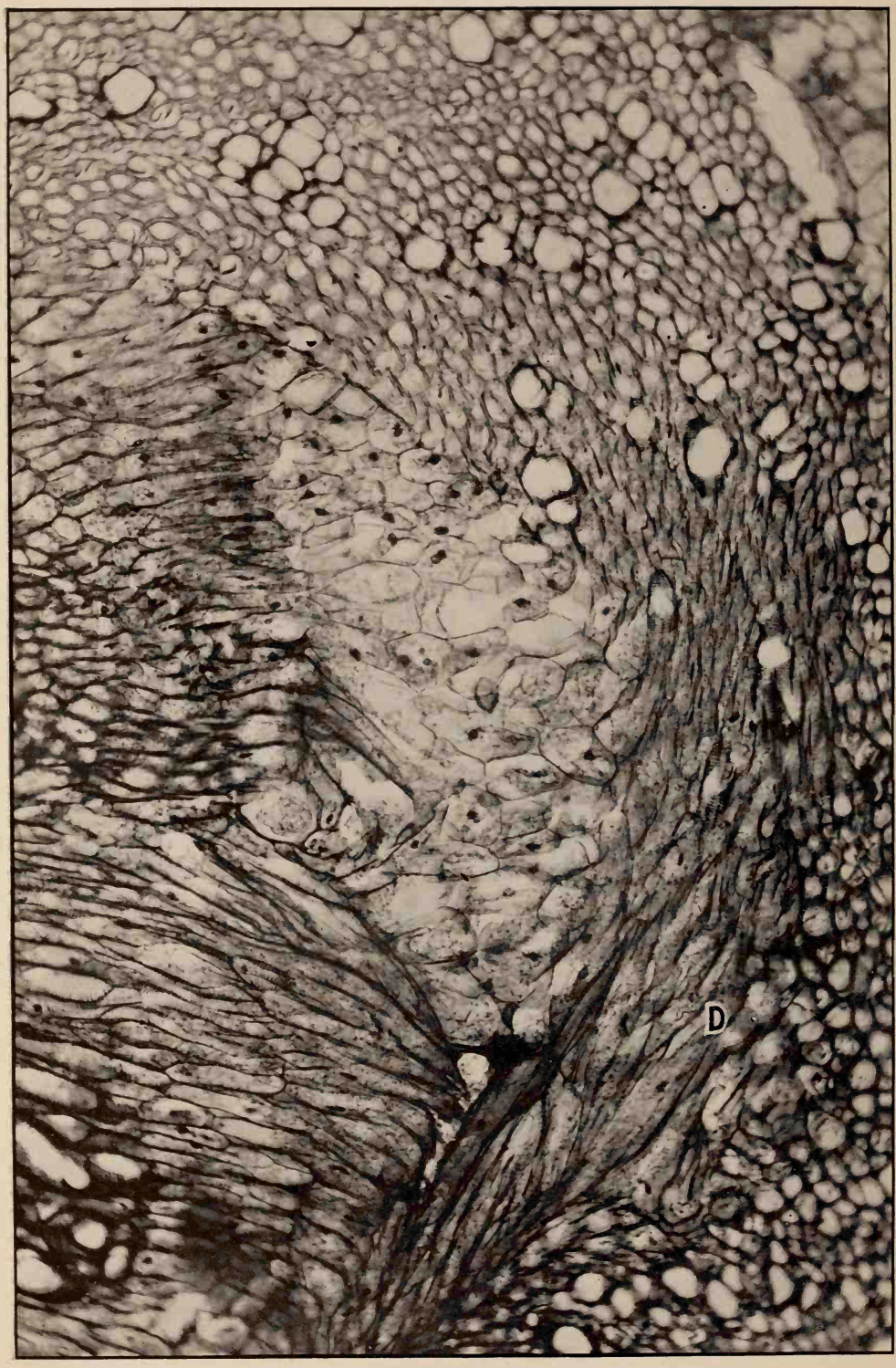
Daisy XIV. Portion of inoculated plant, showing primary stem tumor and secondary leaf tumors (p. 40).



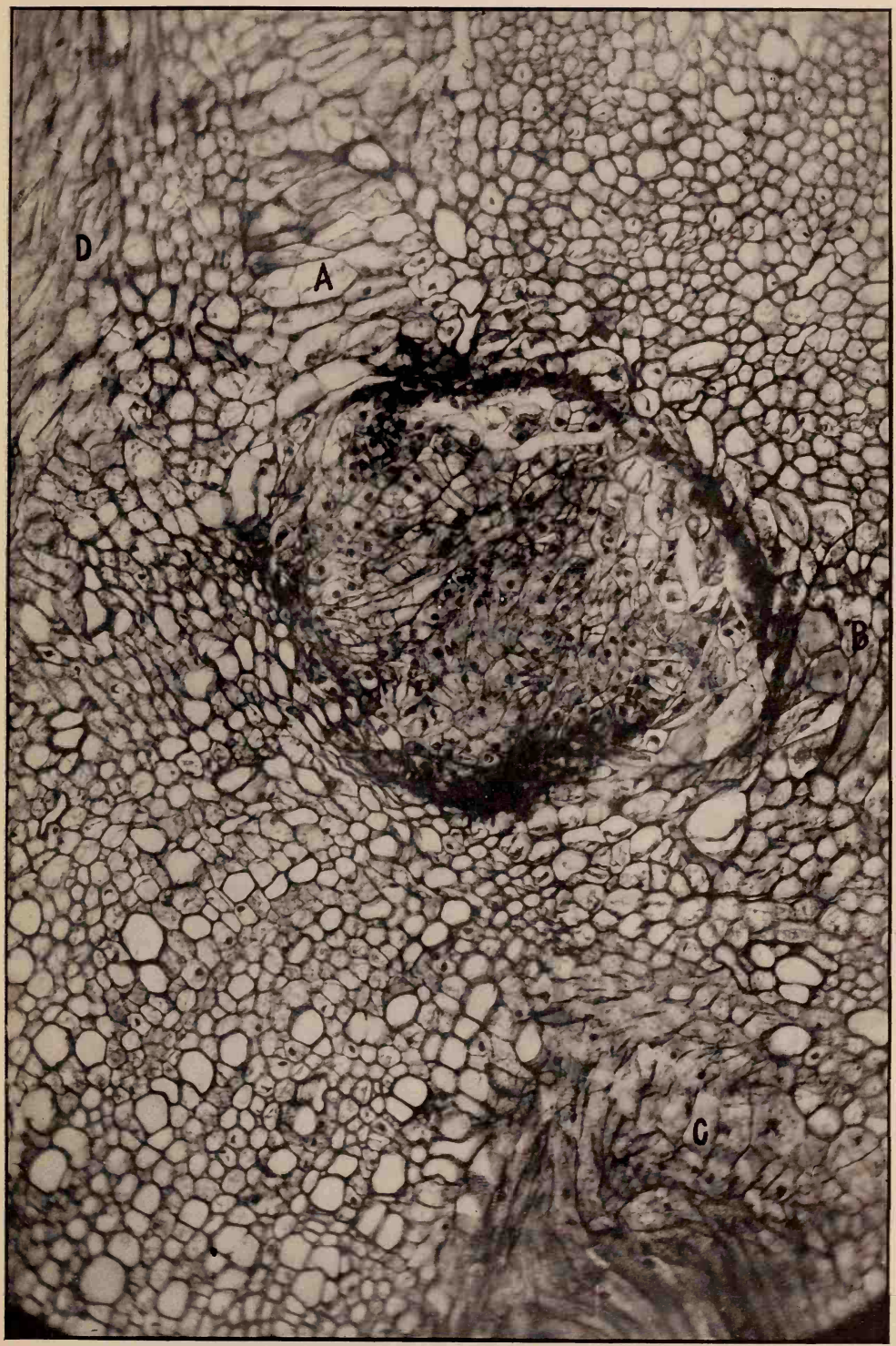
Daisy XIV. Cross section of petiole C, showing secondary tumor with stem structure. What remains of the normal tissue is above and at the left (p. 40).



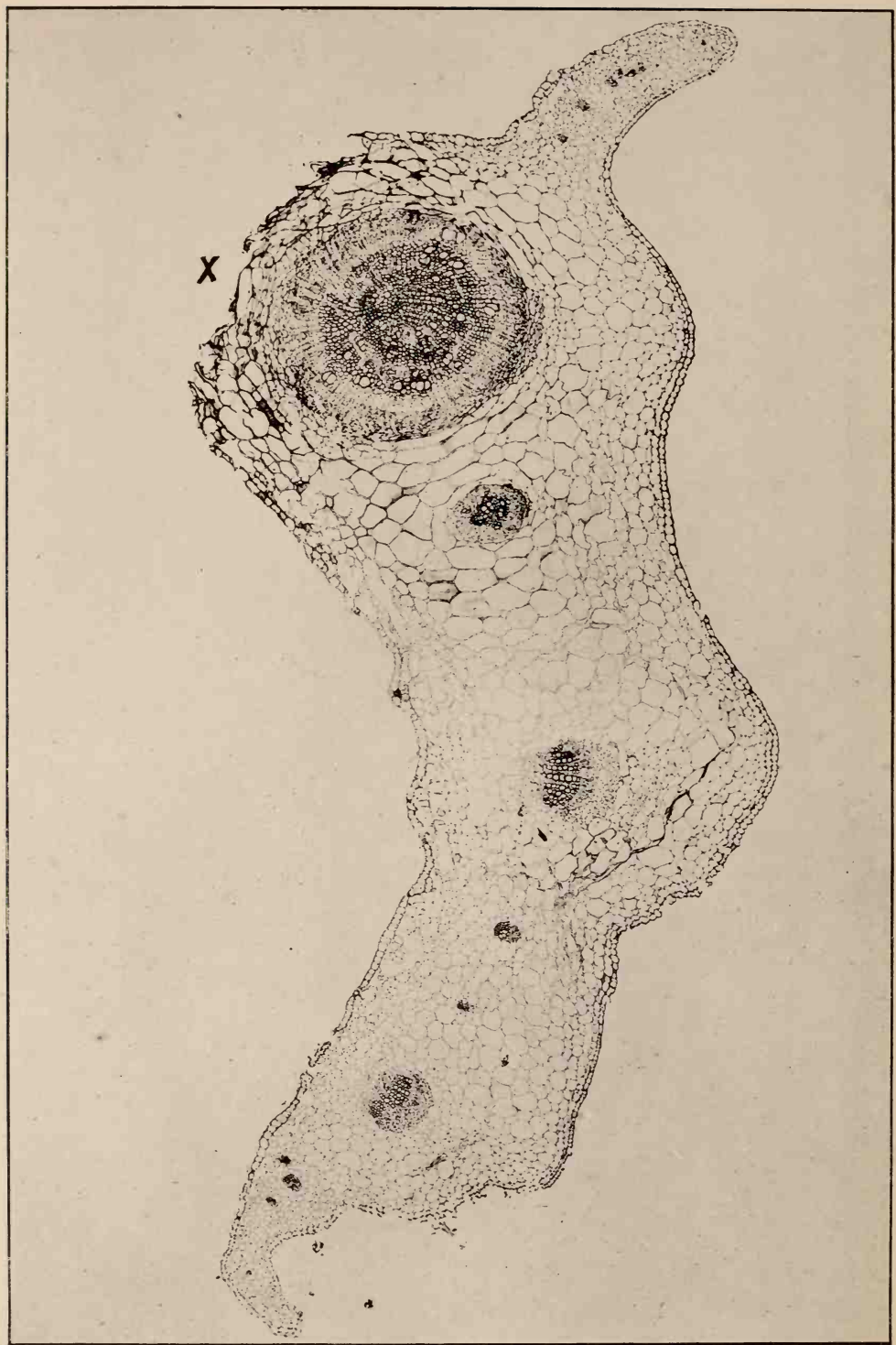
Daisy XIV. Cross section of petiole C, showing abnormal medullary ray with normal and abnormal tracheids (p. 41).



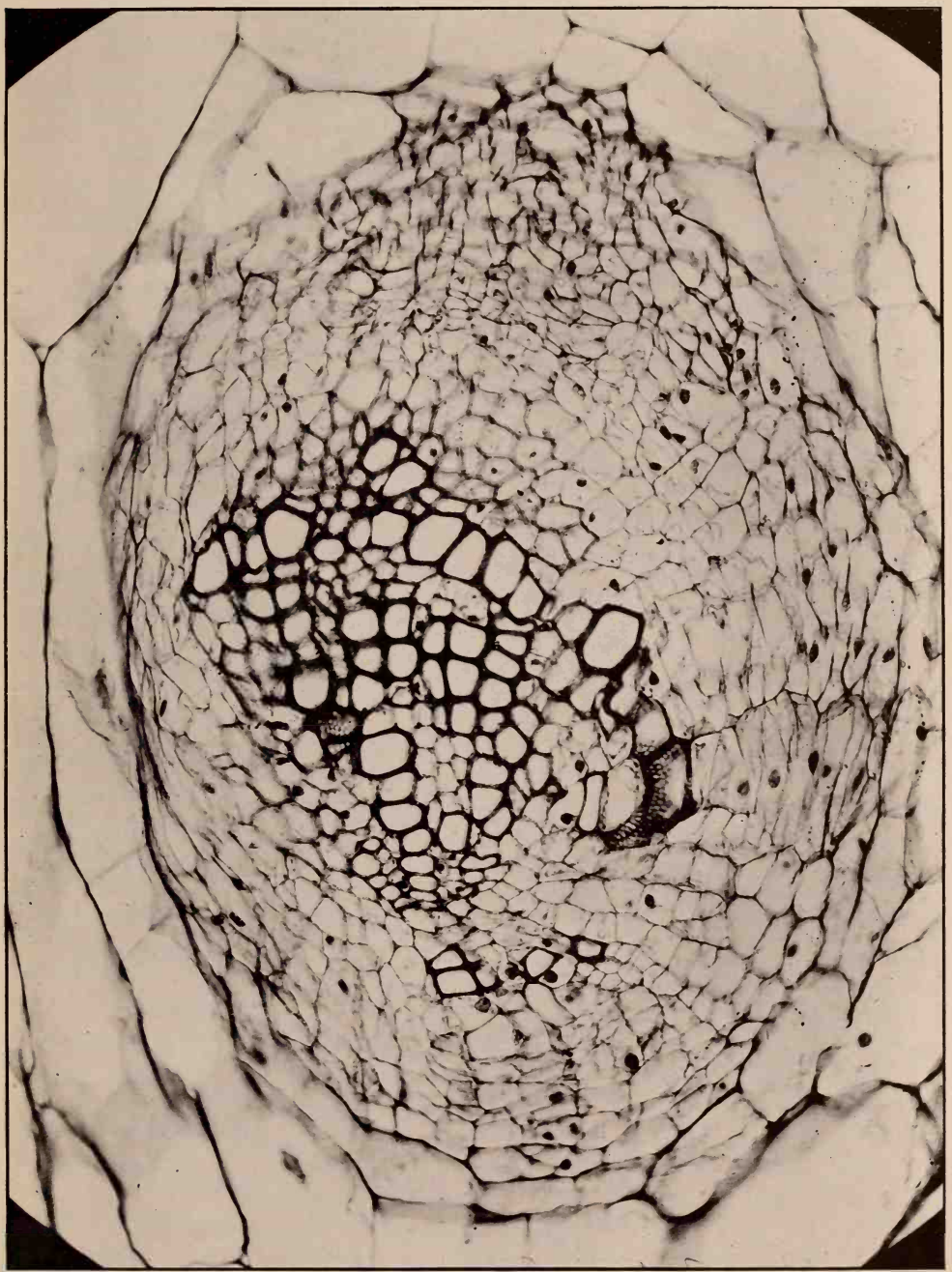
Daisy XIV. Hypertrophied cells in tumor in petiole C (p. 41).



Daisy XIV. Cross section of the tumor-strand in petiole C (p. 41).

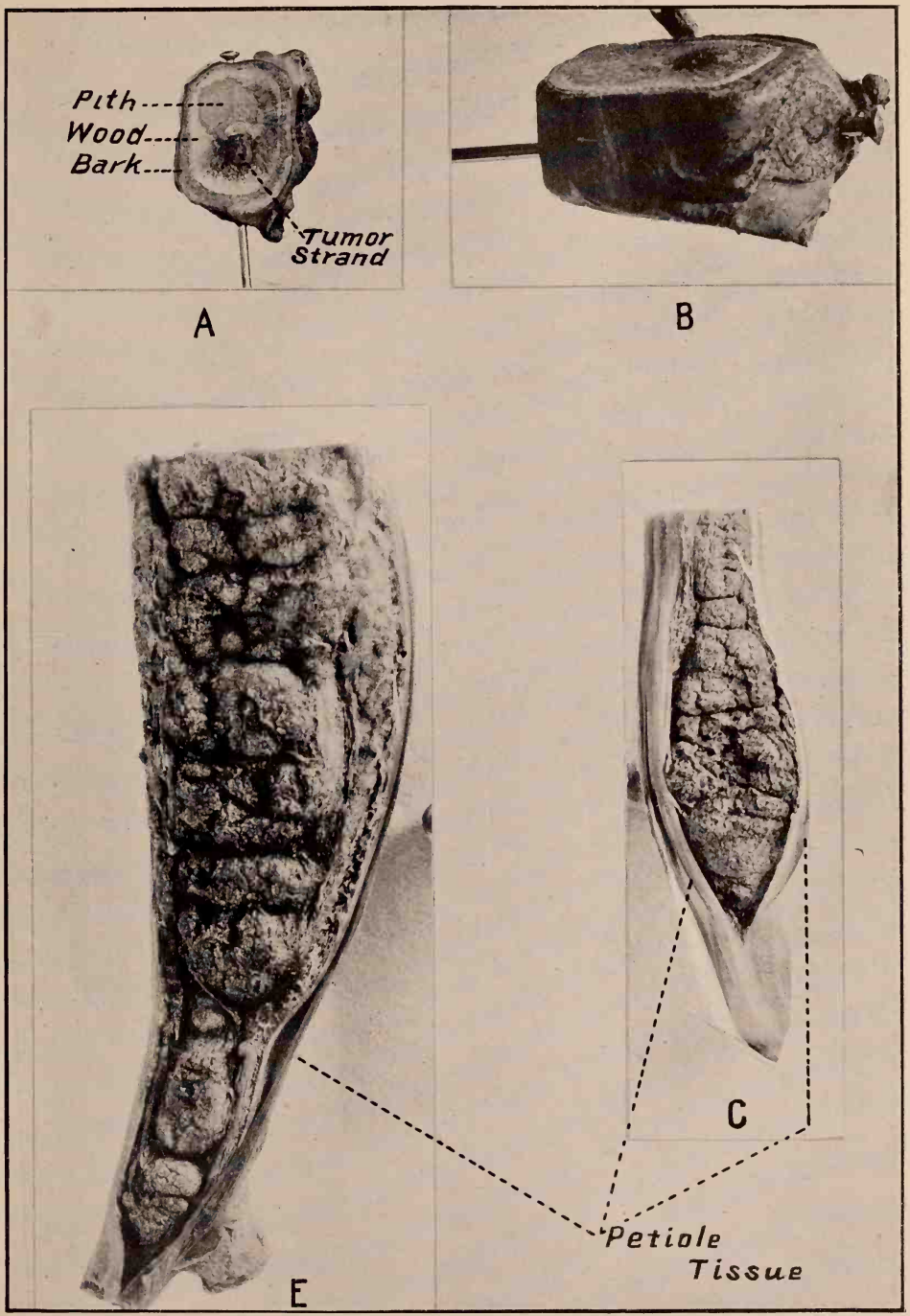


Daisy XIV. Cross section of petiole B, showing a secondary tumor with stem structure at X in a lateral leaf-trace (p. 41).

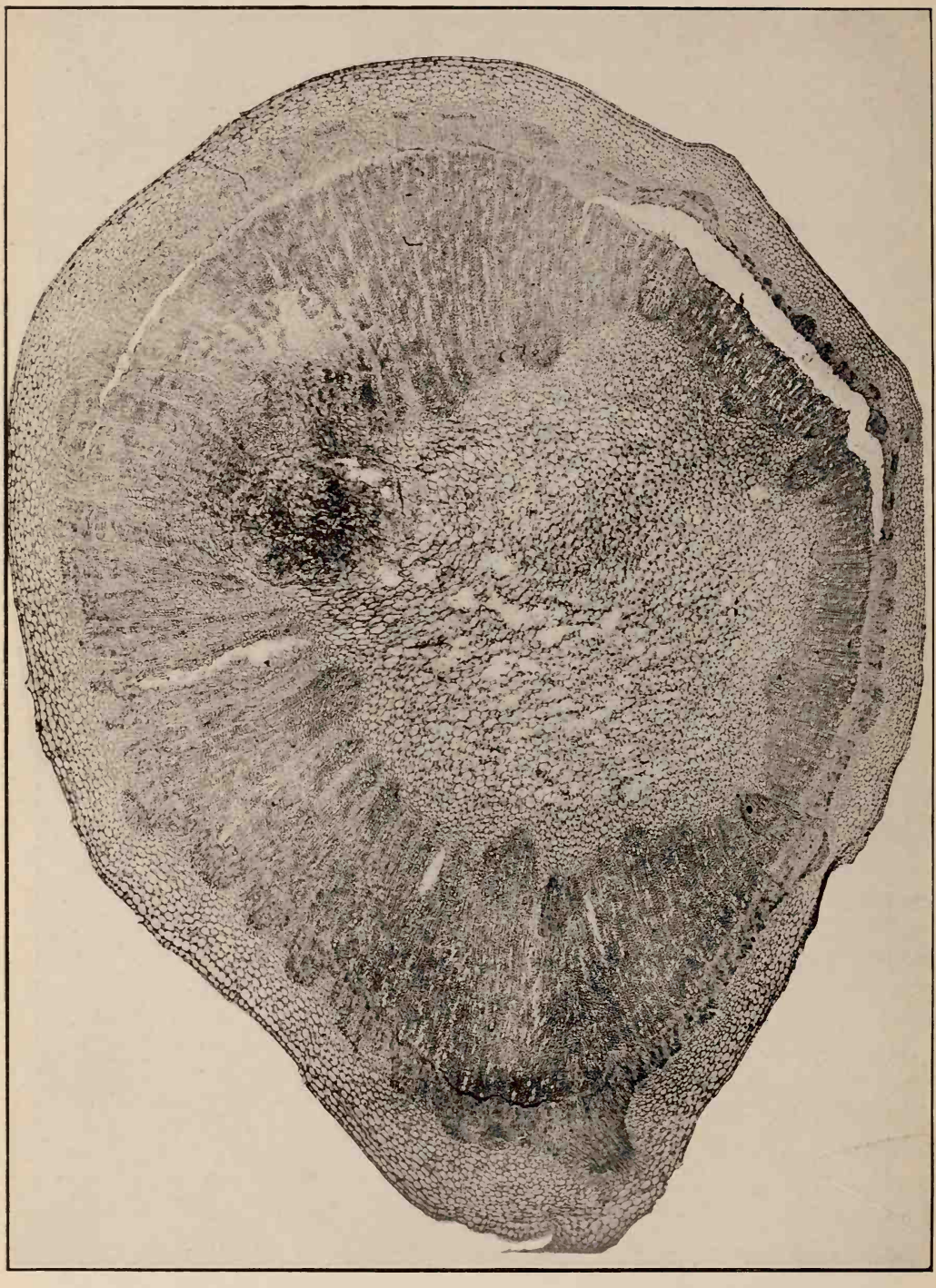


Daisy XIV. Petiole B, showing disturbance in leaf-trace next below X of Plate LIX (p. 42).

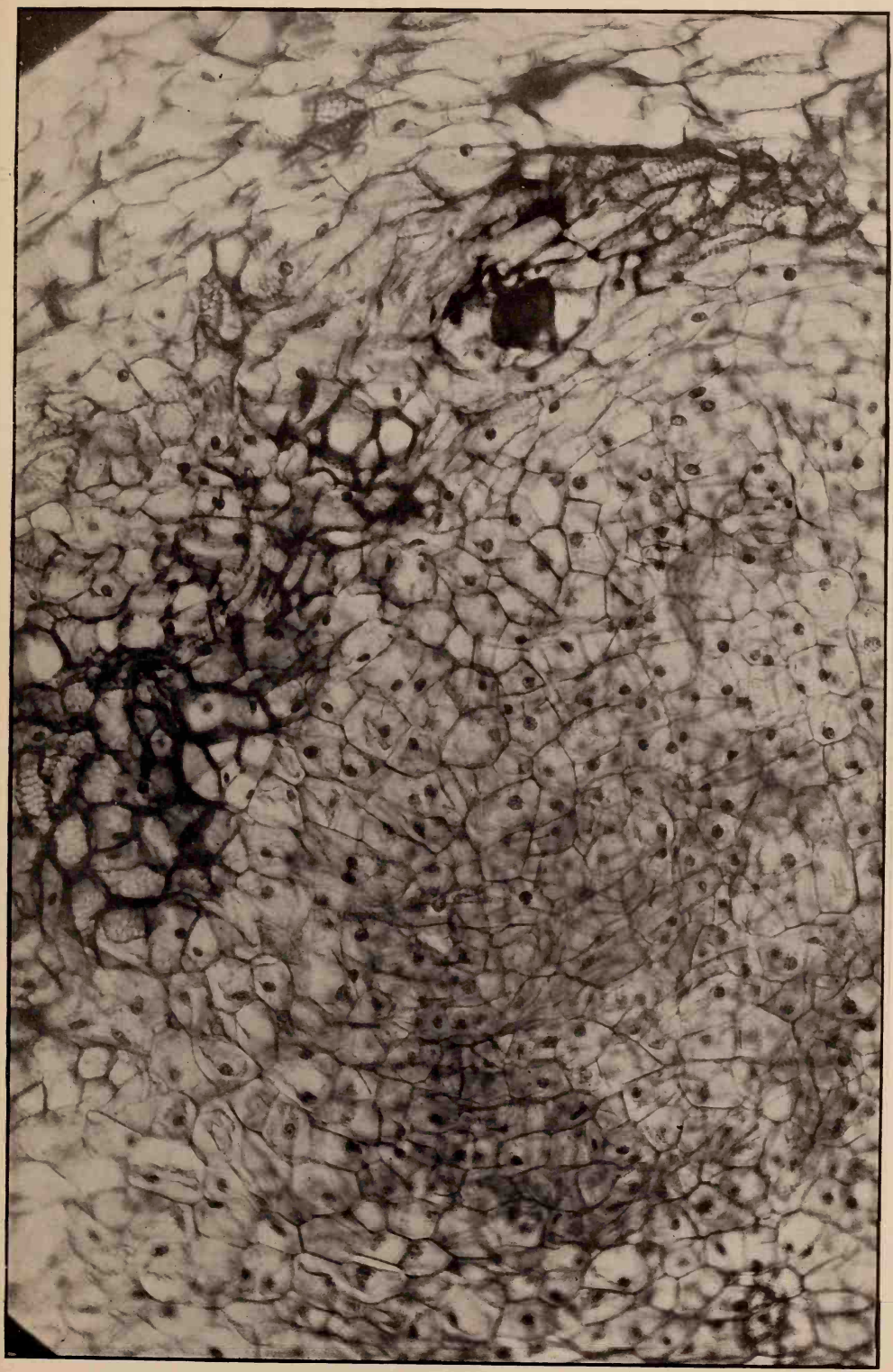
Daisy XVI. Inoculated plant, showing primary stem tumor and secondary leaf tumors (p. 42).



Daisy XVI. Cross section of stem between tumors, showing protrusion of tumor-strand; also petioles enlarged to show how the secondary tumor reaches the surface, i. e., by splitting open the overlying tissues (p. 42).



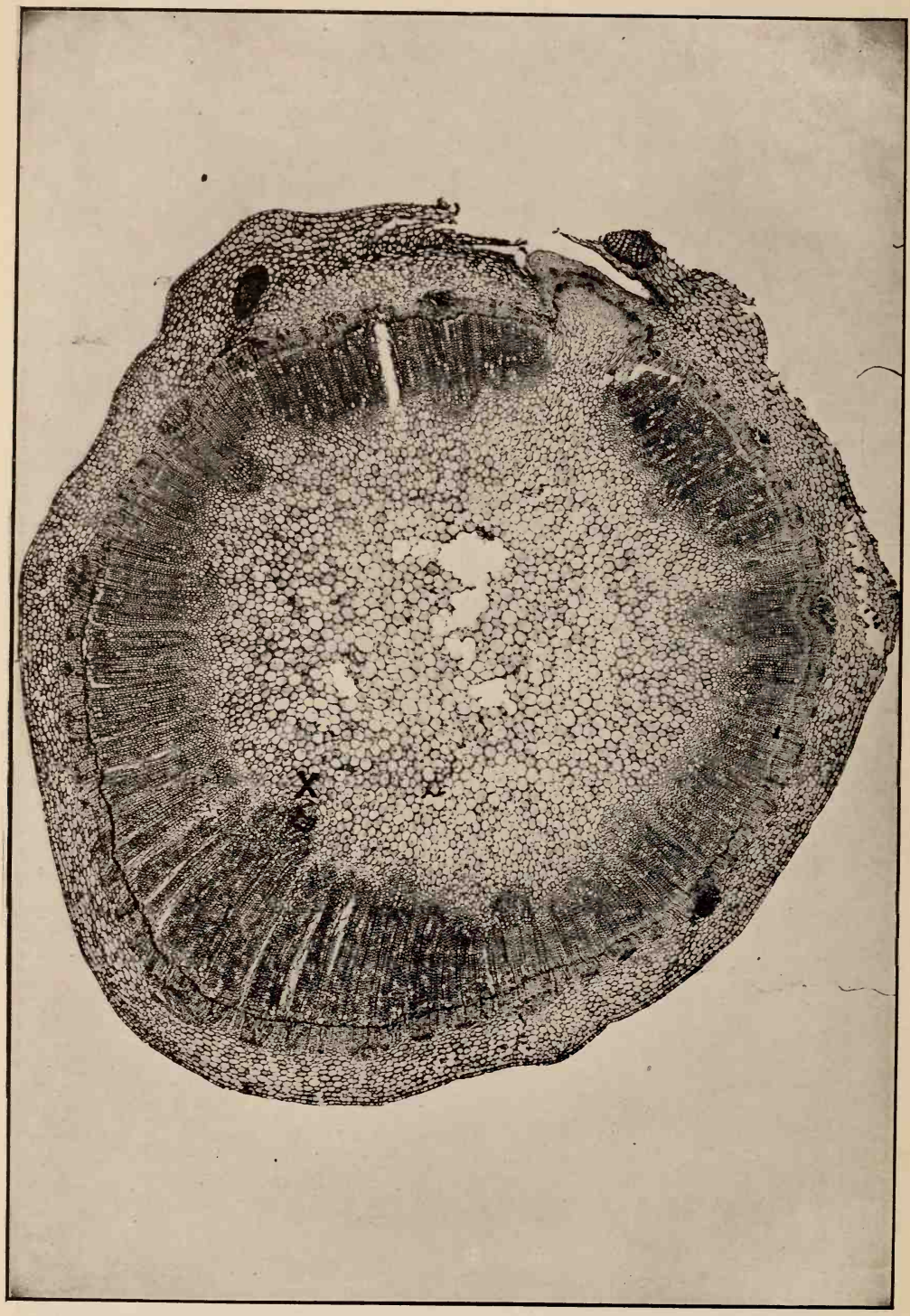
Daisy XVI. Magnified cross section of stem between tumors; tumor-strand at left; wood cylinder thickened in three-fourths of its circumference (p. 43).



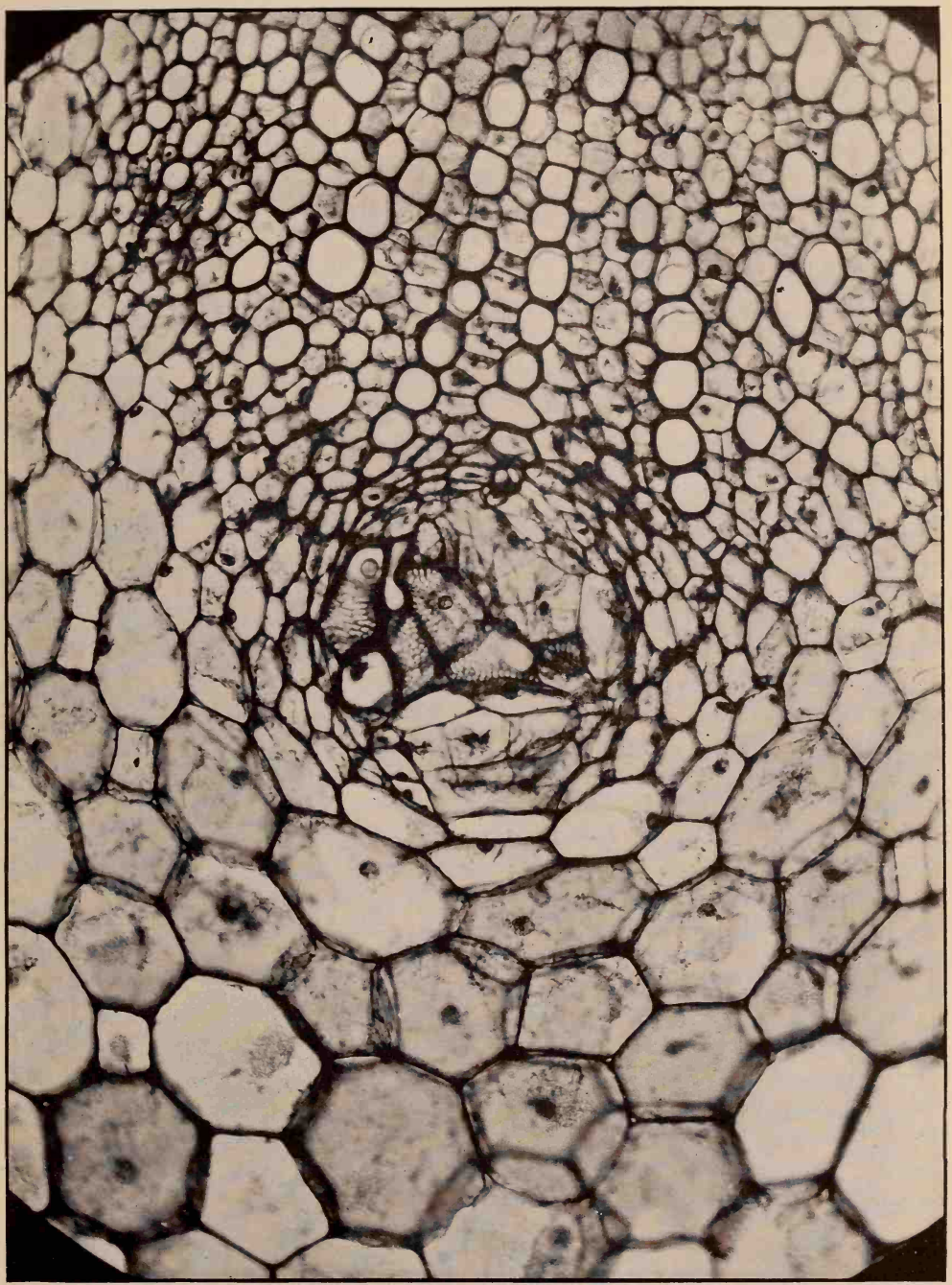
Daisy XVI. Margin of small portion of tumor-strand from Plate LXIII (p. 43).

Daisy XVI. Tumor-strand and stroma of twisted tracheids, spirals at S, with remainder of them in direction of the arrow (p. 43).

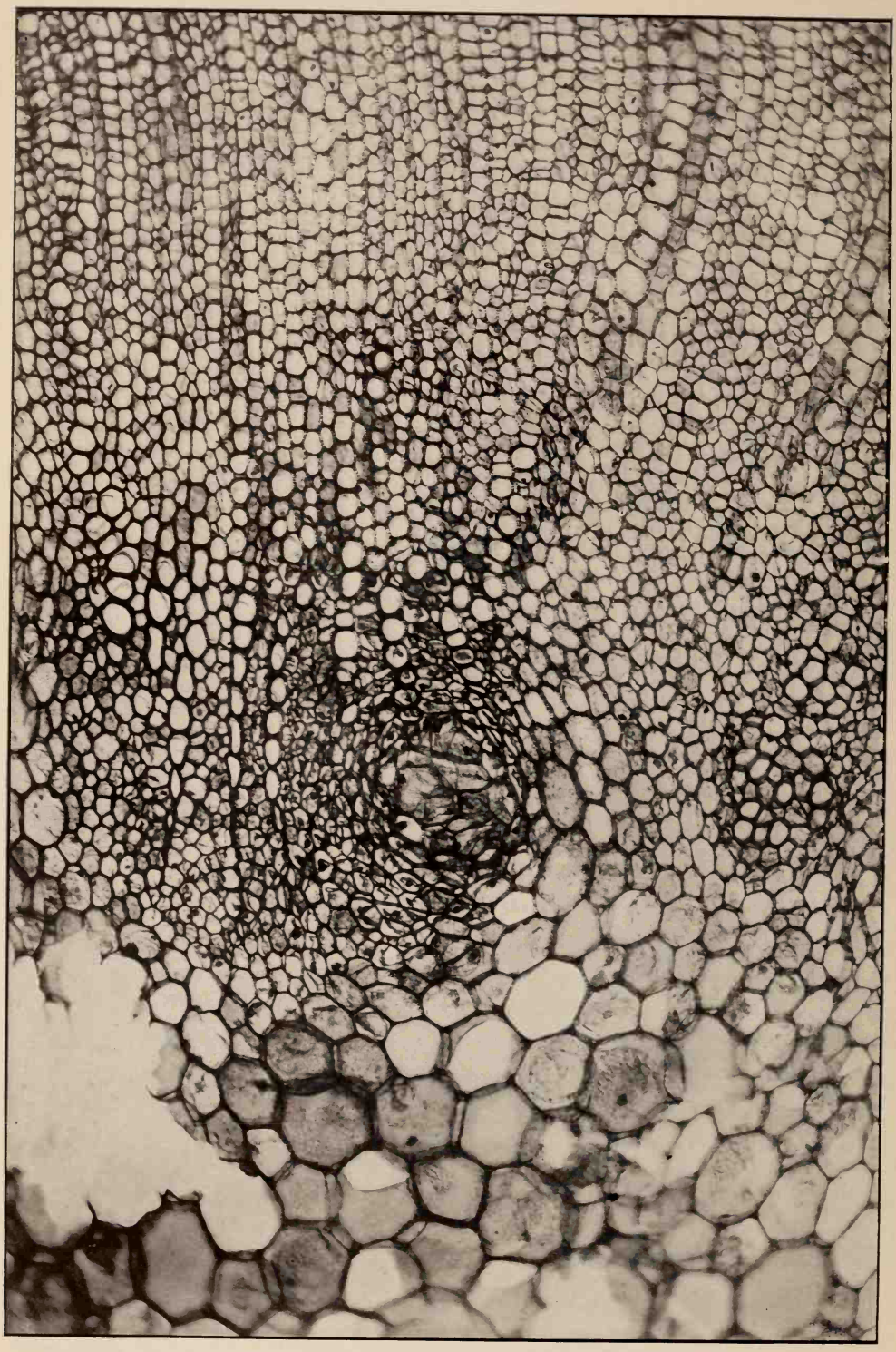
Daisy XVII. Inoculated plant, showing primary stem tumor and secondary leaf tumors (p. 43).



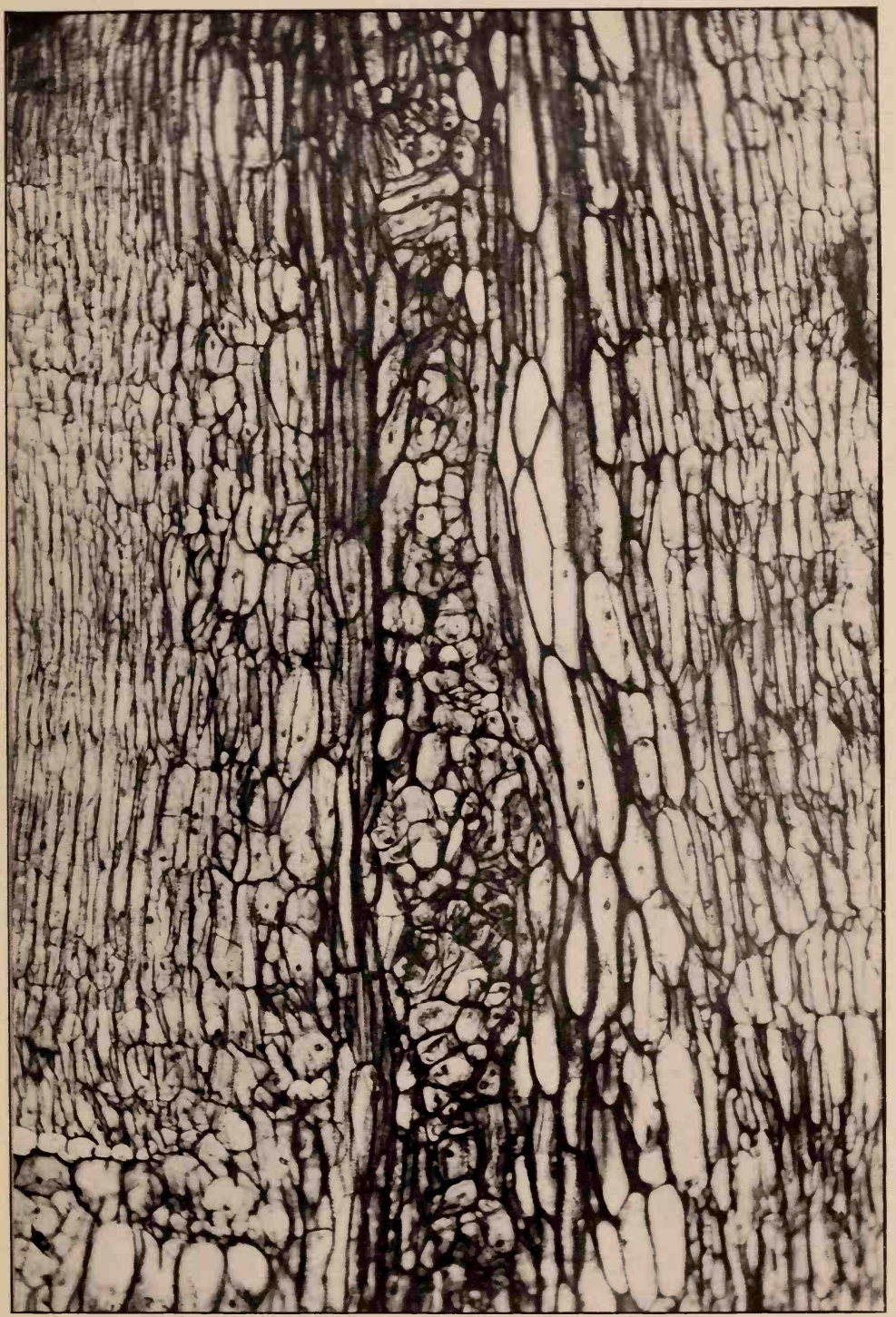
Daisy XVII. Cross section of stem between tumors, showing a small tumor-strand at X (p. 44).



Daisy XVII. Portion of Plate LXVII enlarged, showing tracheids developed in the tumor-strand; the vessels immediately above are spirals (p. 44).

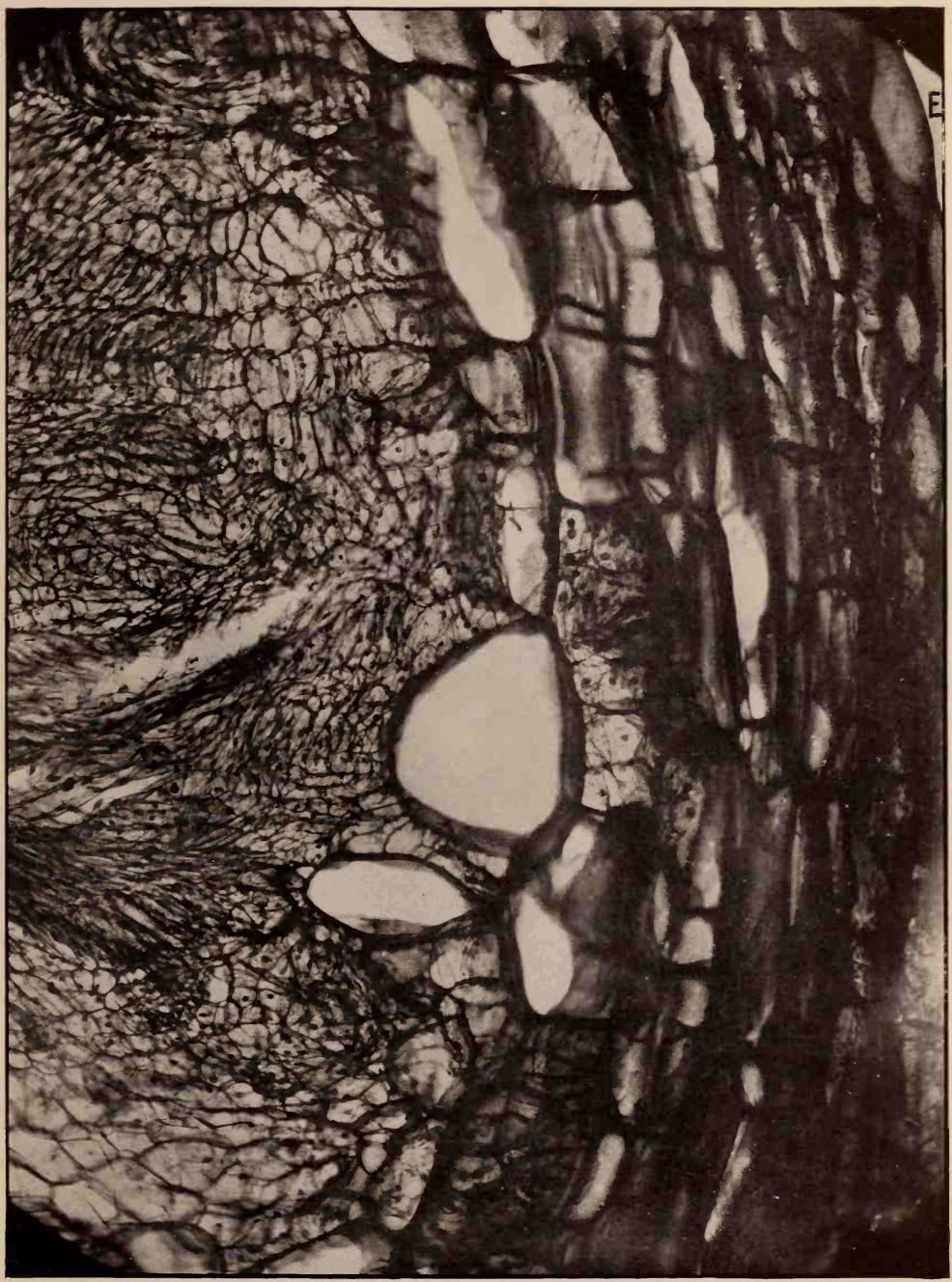


Daisy XVII. Cross section of stem at another level, showing tumor-strand (p. 44).



Daisy XVII. Longitudinal section of petiole C, showing tumor-strand (p. 44).

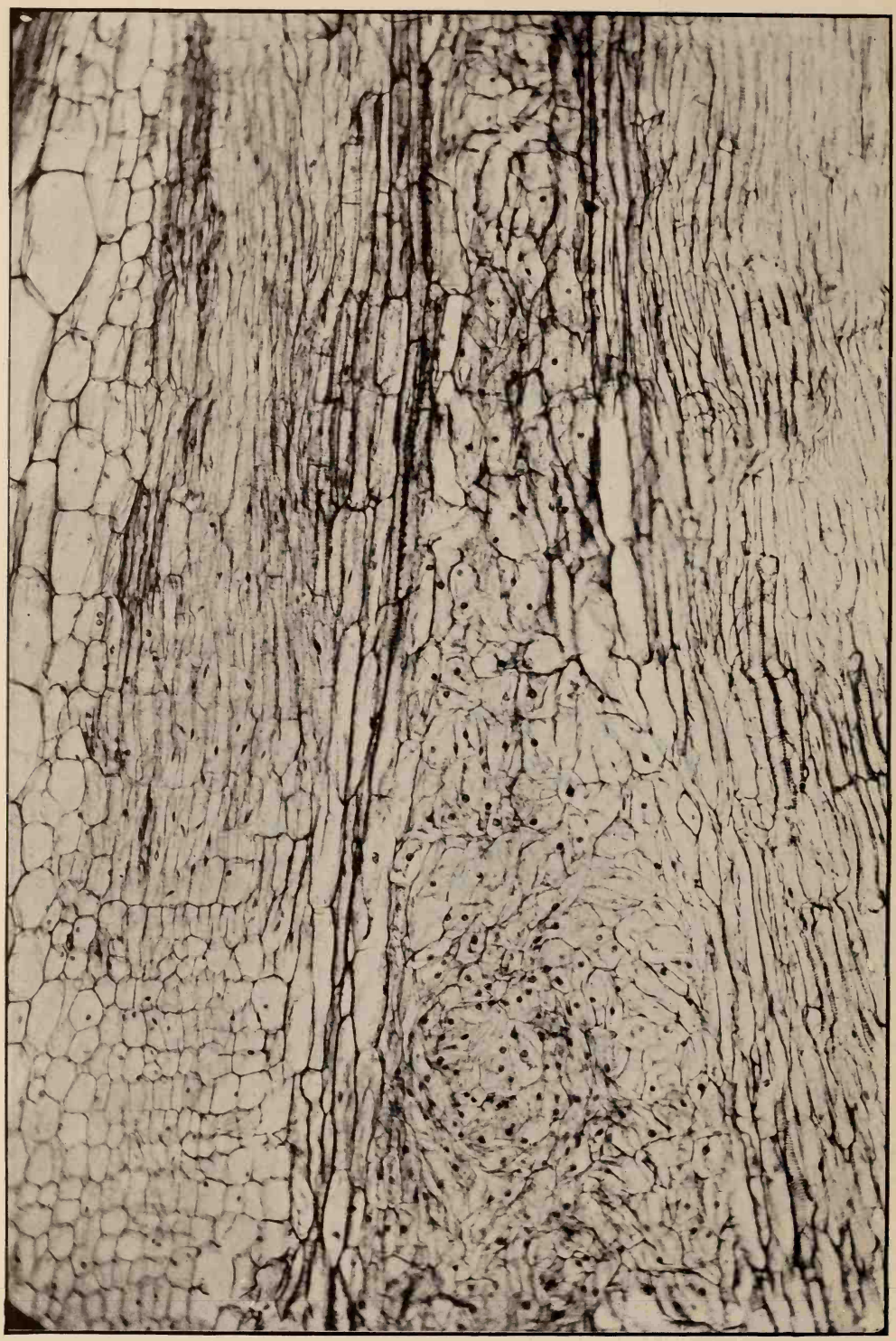
Daisy XVII. Tumor tissue of petiole C with bi- and tri-nucleate cells (p. 45).



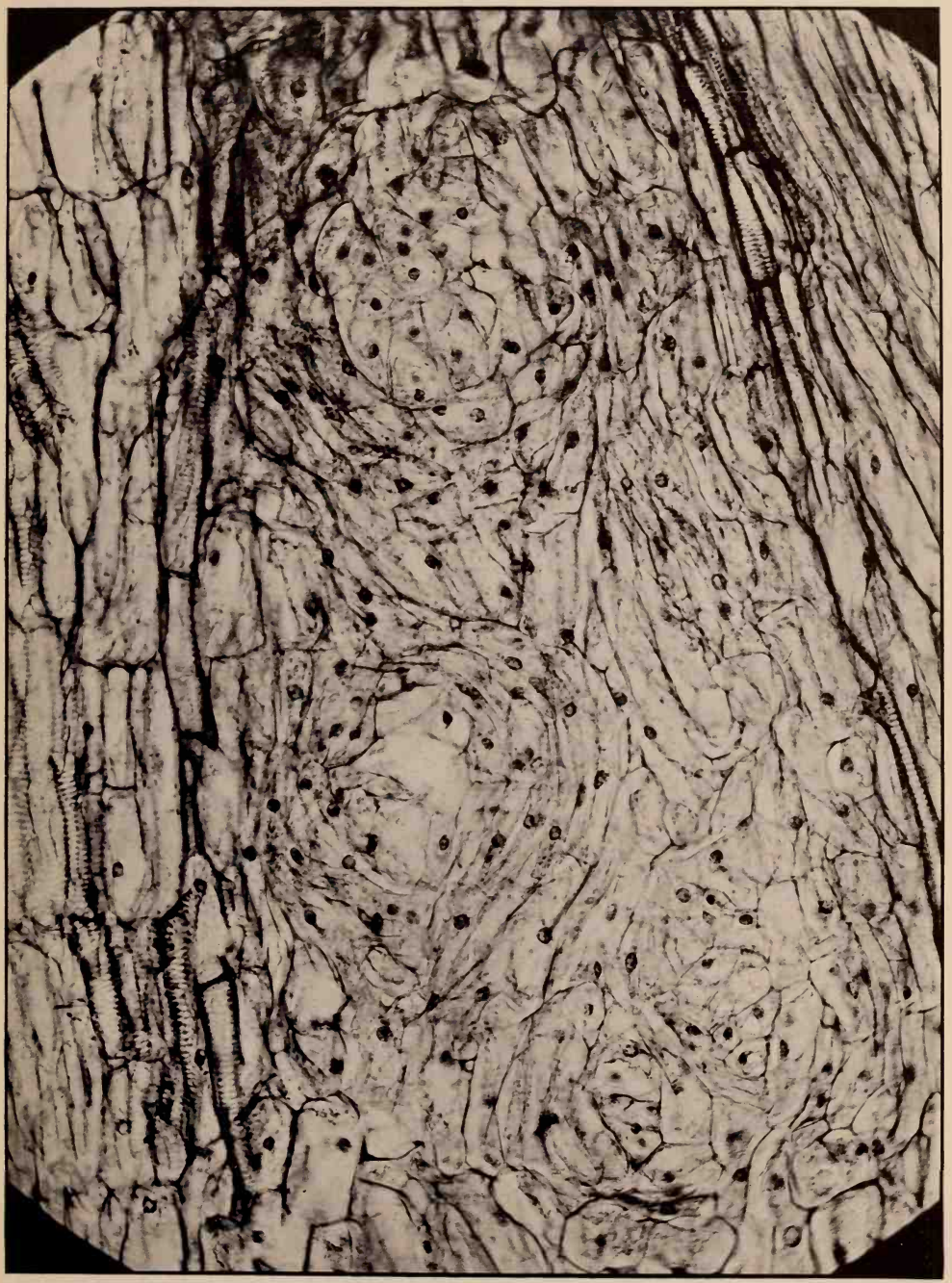
Daisy XVII. Margin of secondary tumor (petiole C) showing infiltration (p. 45).

Daisy XVIII. Inoculated plant, showing primary tumor on stem and secondary tumors on leaves (p. 45).

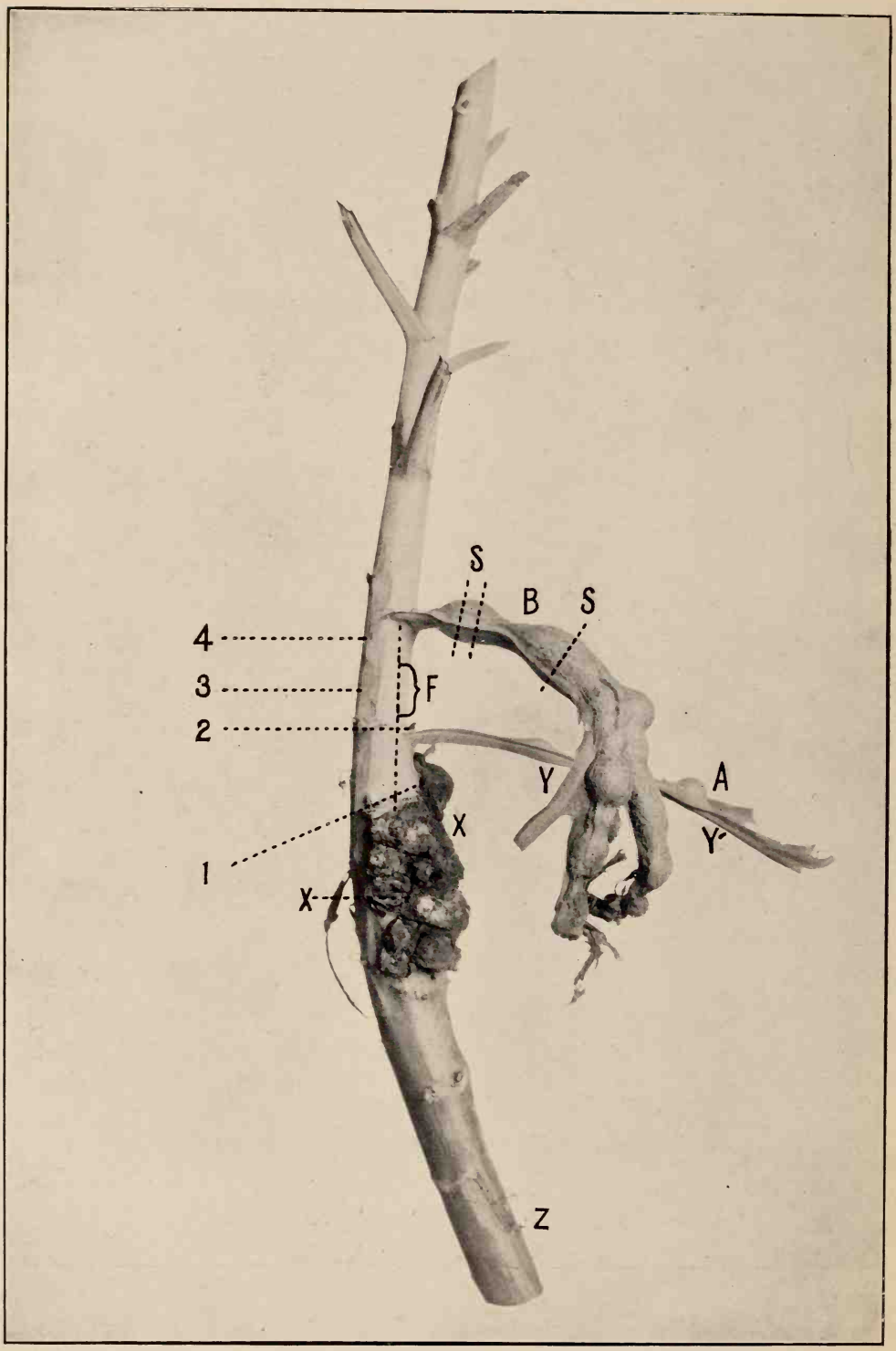
Daisy XVIII. Longitudinal section of petiole A, showing tumor-strand (p. 45).



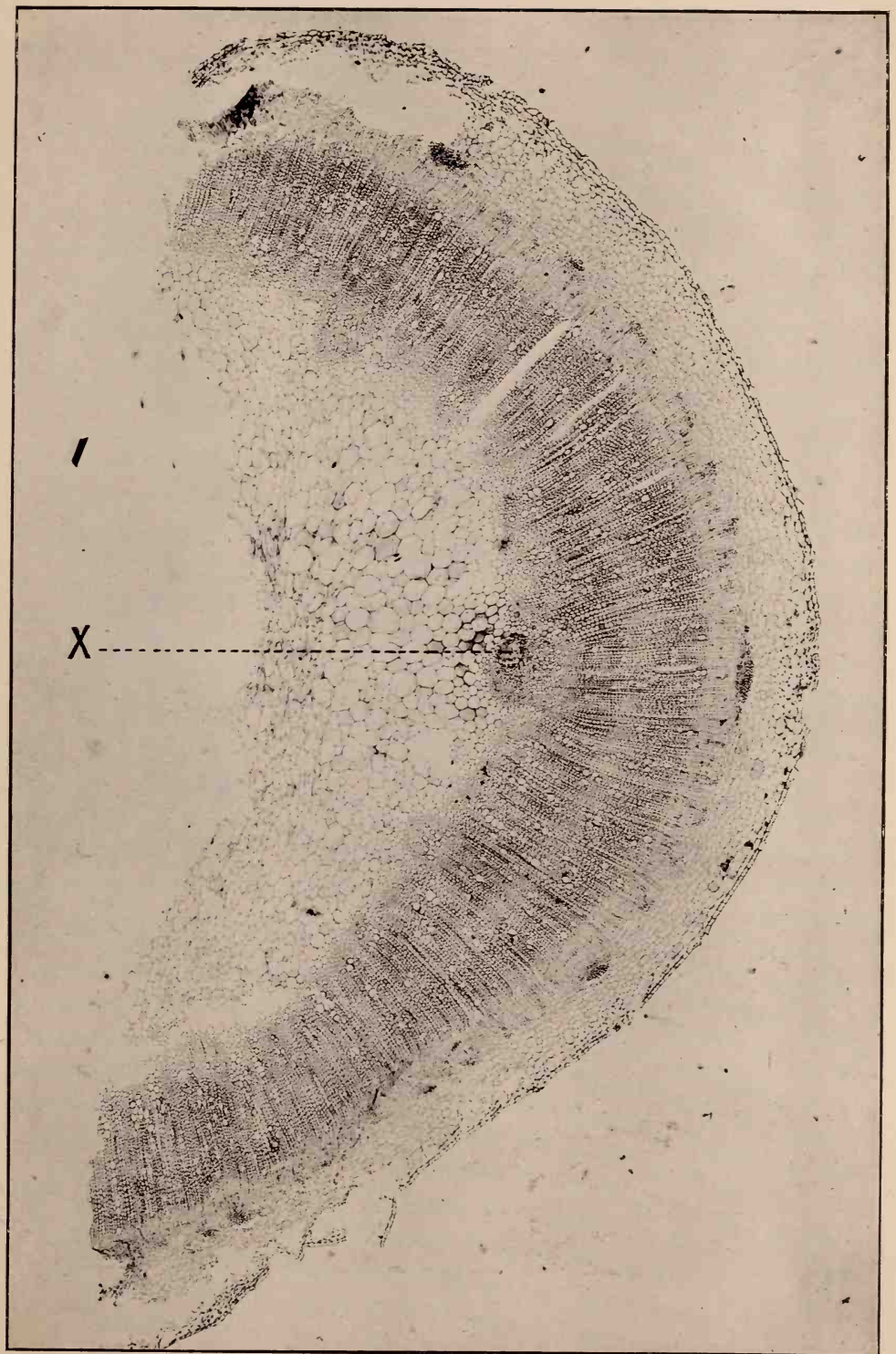
Daisy XVIII. Tumor-strand in petiole A; joins on to Plate LXXIV (p. 46).



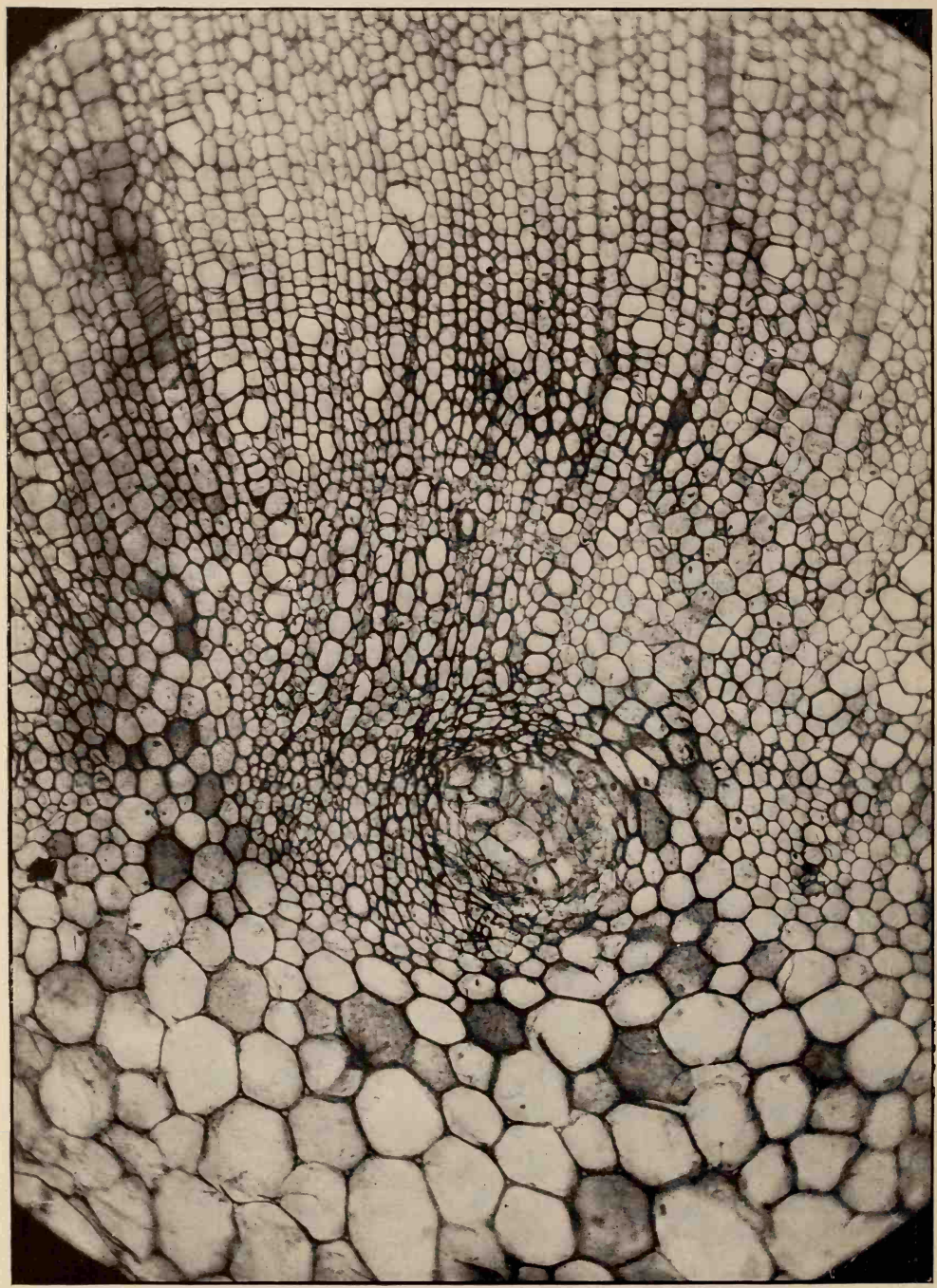
Daisy XVIII. Tumor-strand in petiole A; joins on to Plate LXXV (p. 46).



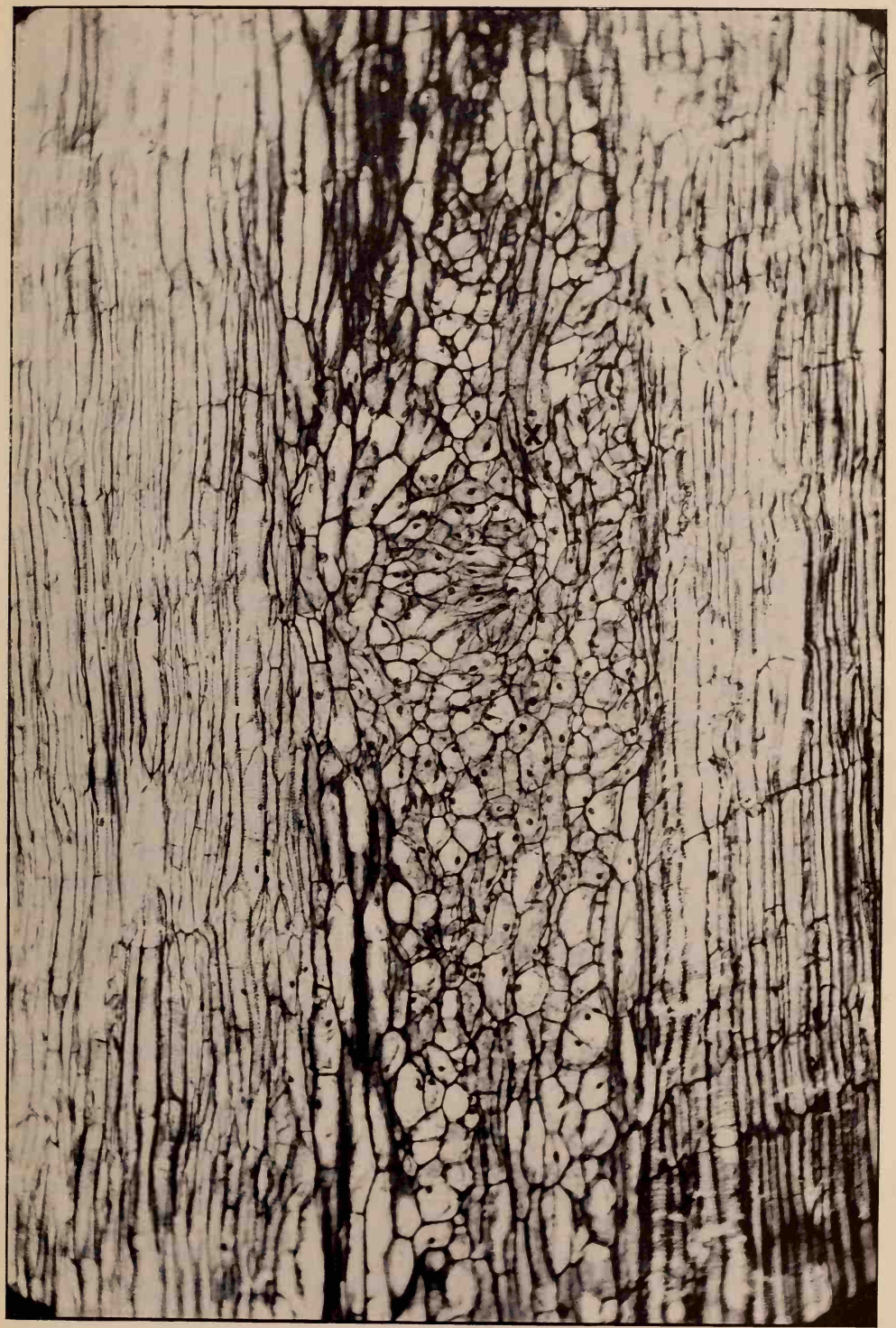
Daisy XIX. Portion of inoculated plant, showing primary stem tumor and secondary leaf tumors (p. 46).



Daisy XIX. Cross section of stem between tumors, showing a tumor-strand at X (p. 46).

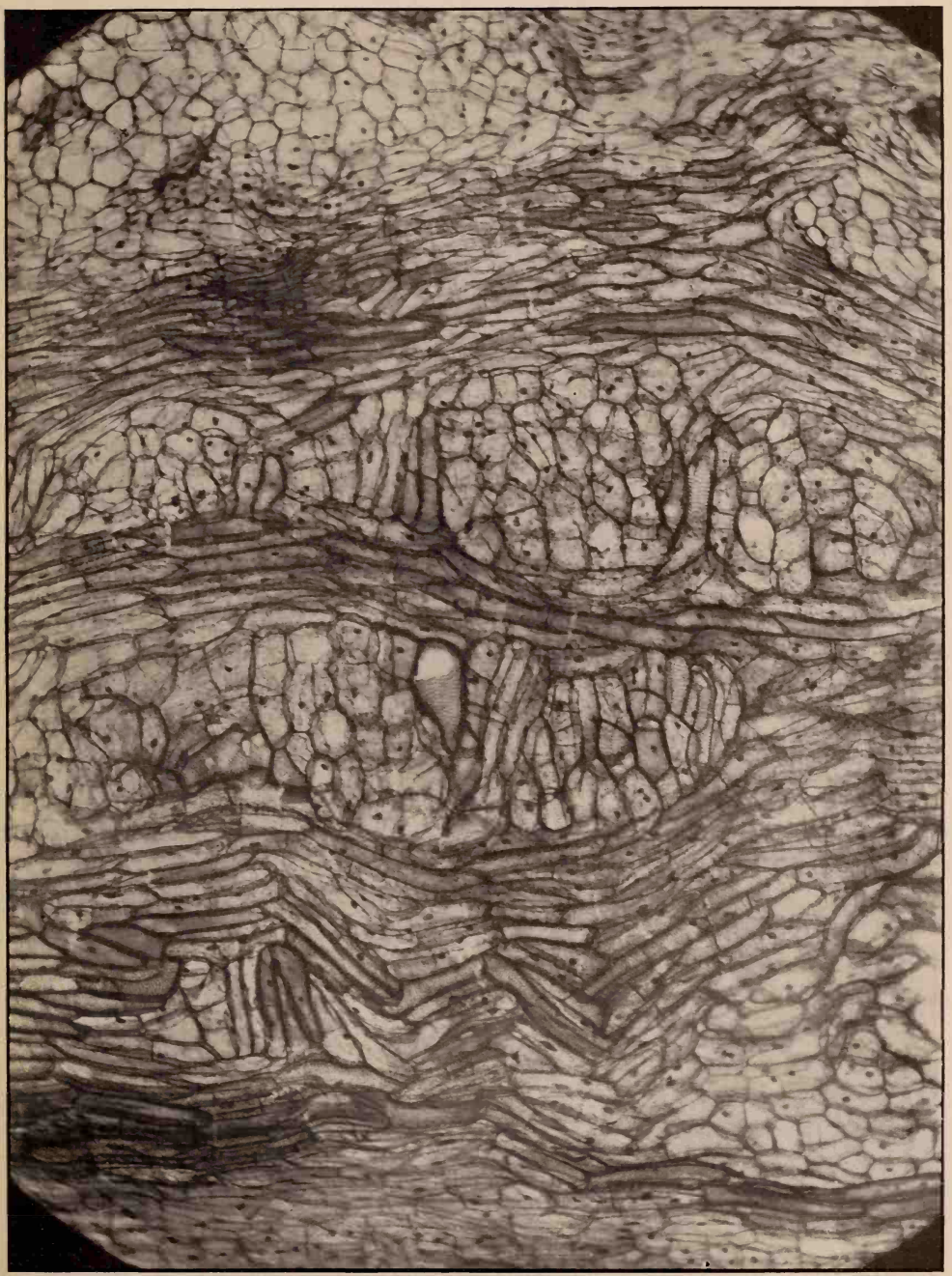


Daisy XIX. A part of Plate LXXVIII, showing tumor-strand region in detail; wood above, pith below (p. 46).

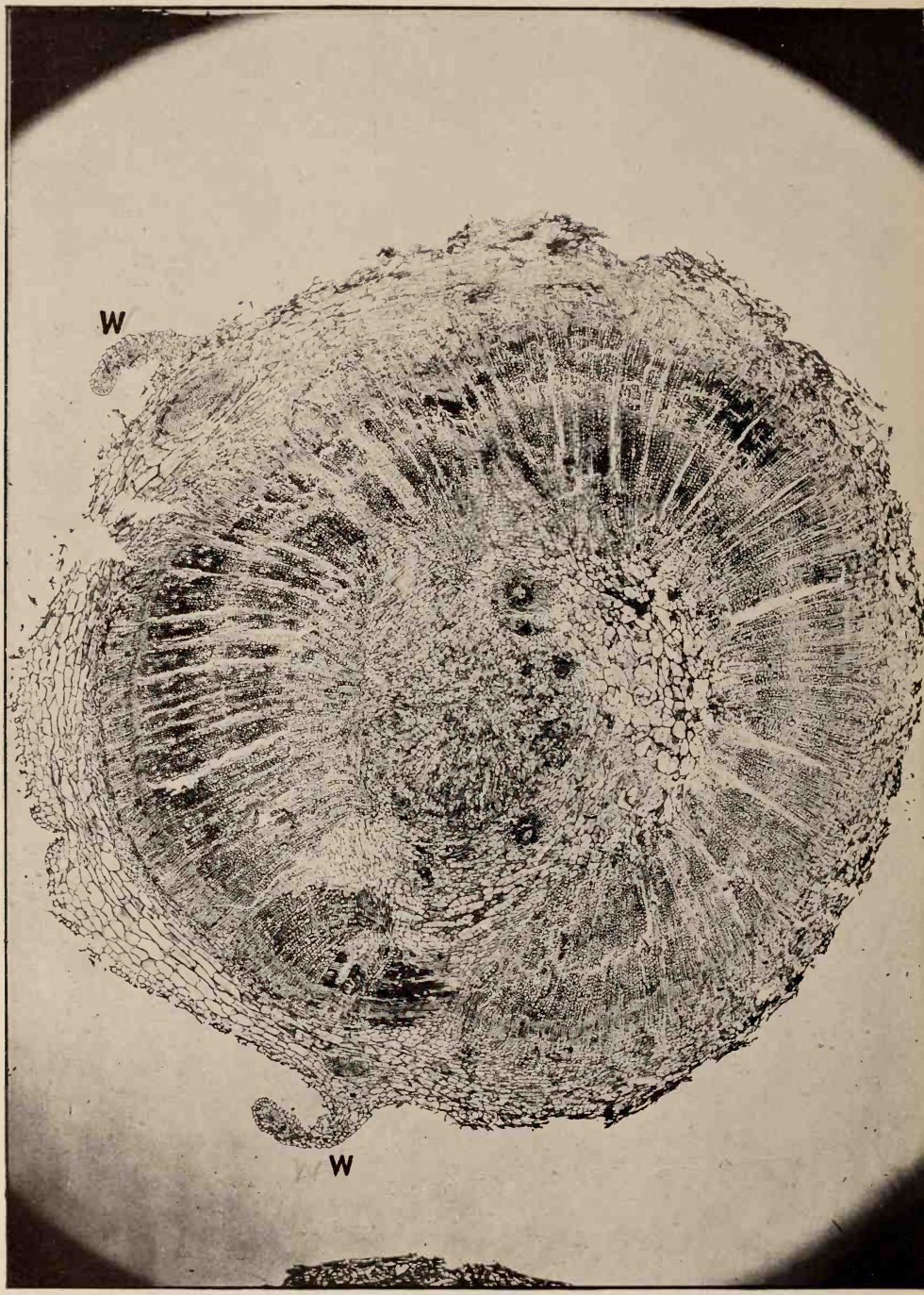


Daisy XIX. Longitudinal section of base of petiole A, showing tumor-strand (p. 47).

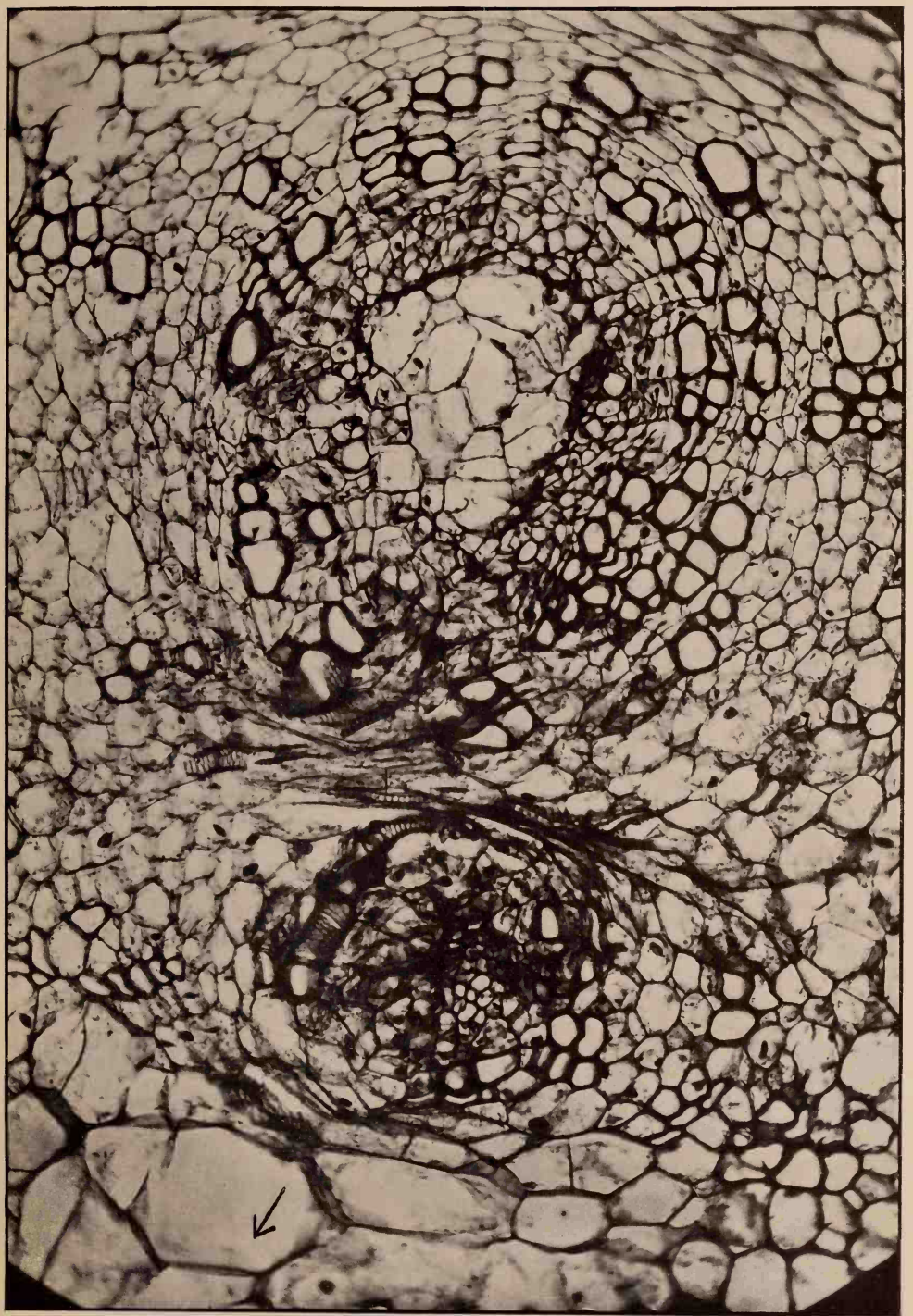
Daisy XIX. Longitudinal section of middle part of petiole A, between tumors (p. 47).



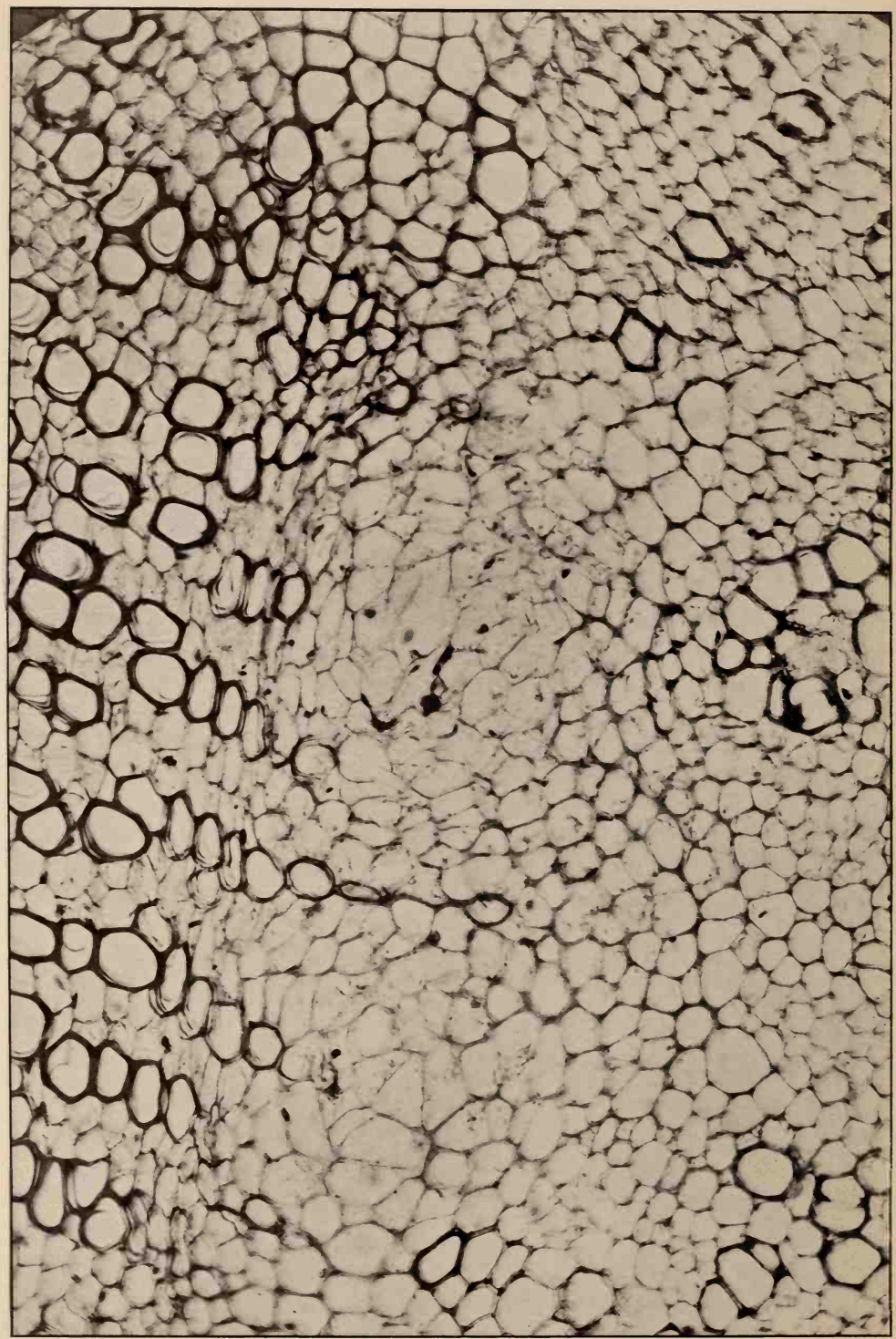
Daisy XIX. Tumor at Y on petiole A, showing the supporting stroma of tracheids (p. 47).



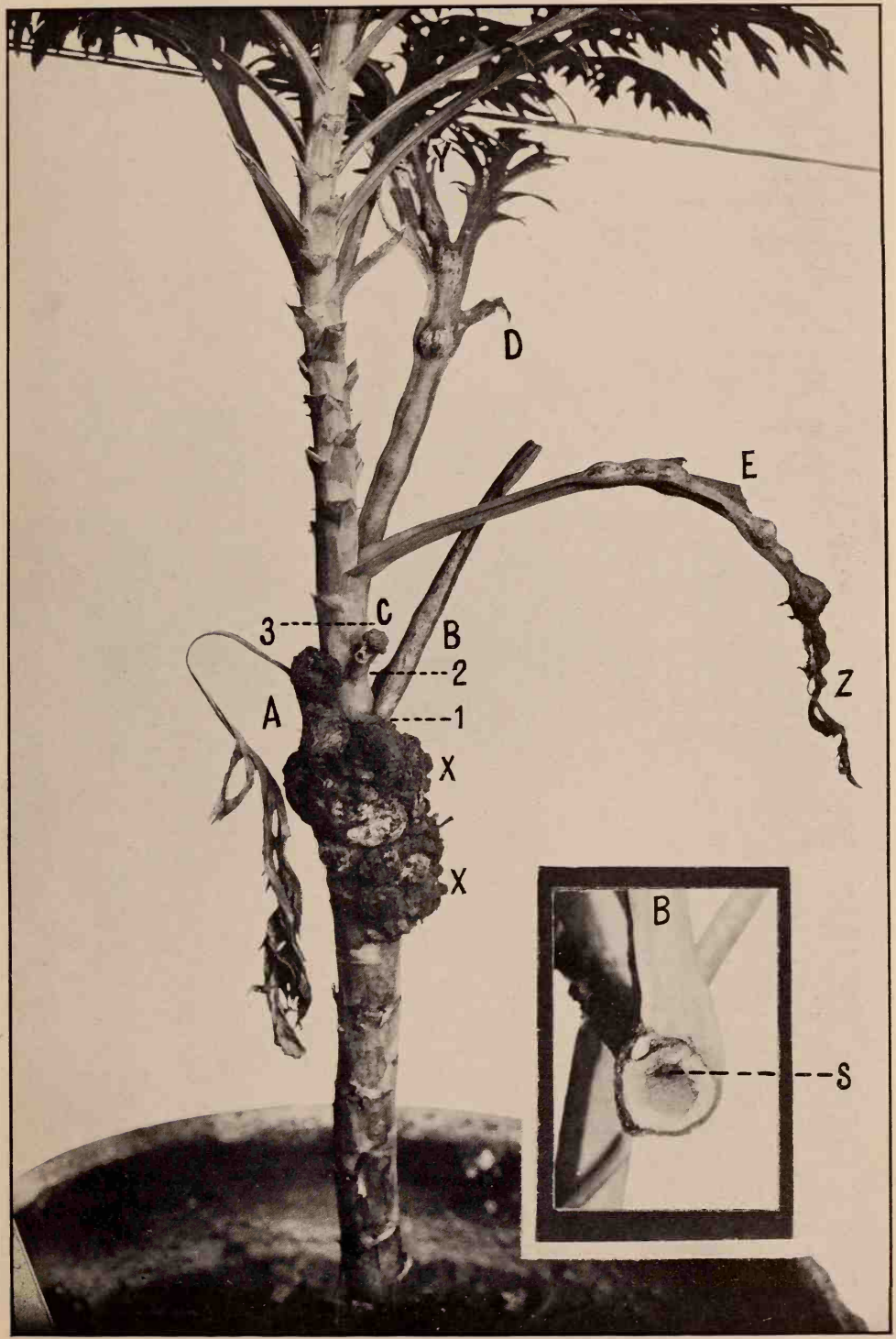
Daisy XIX. Cross section of secondary tumor in petiole B, showing stem structure and coarse-cell inclusions (p. 47).



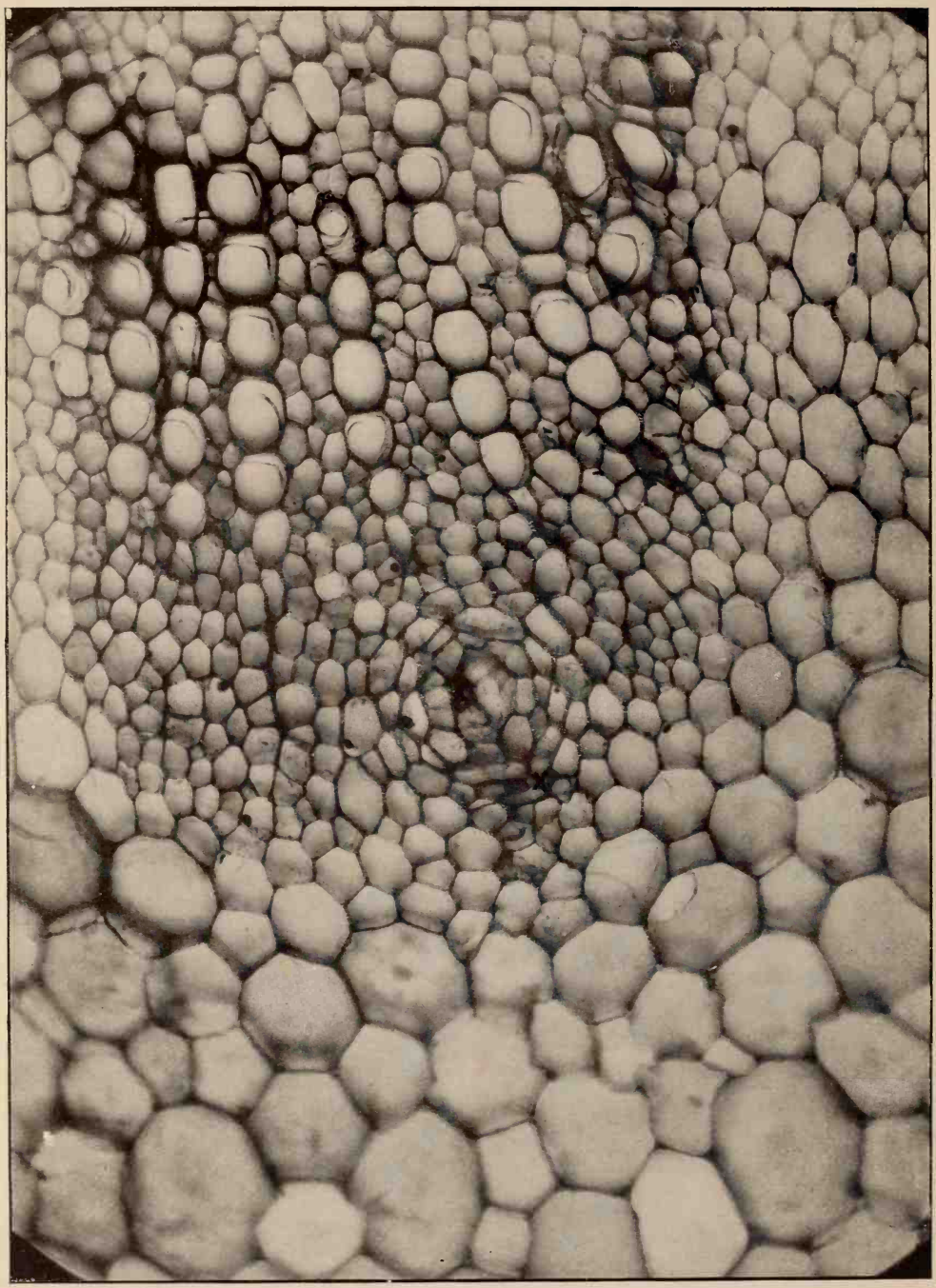
Daisy XIX. Cross section of petiole B, showing whorls in tumor tissue (p. 48).



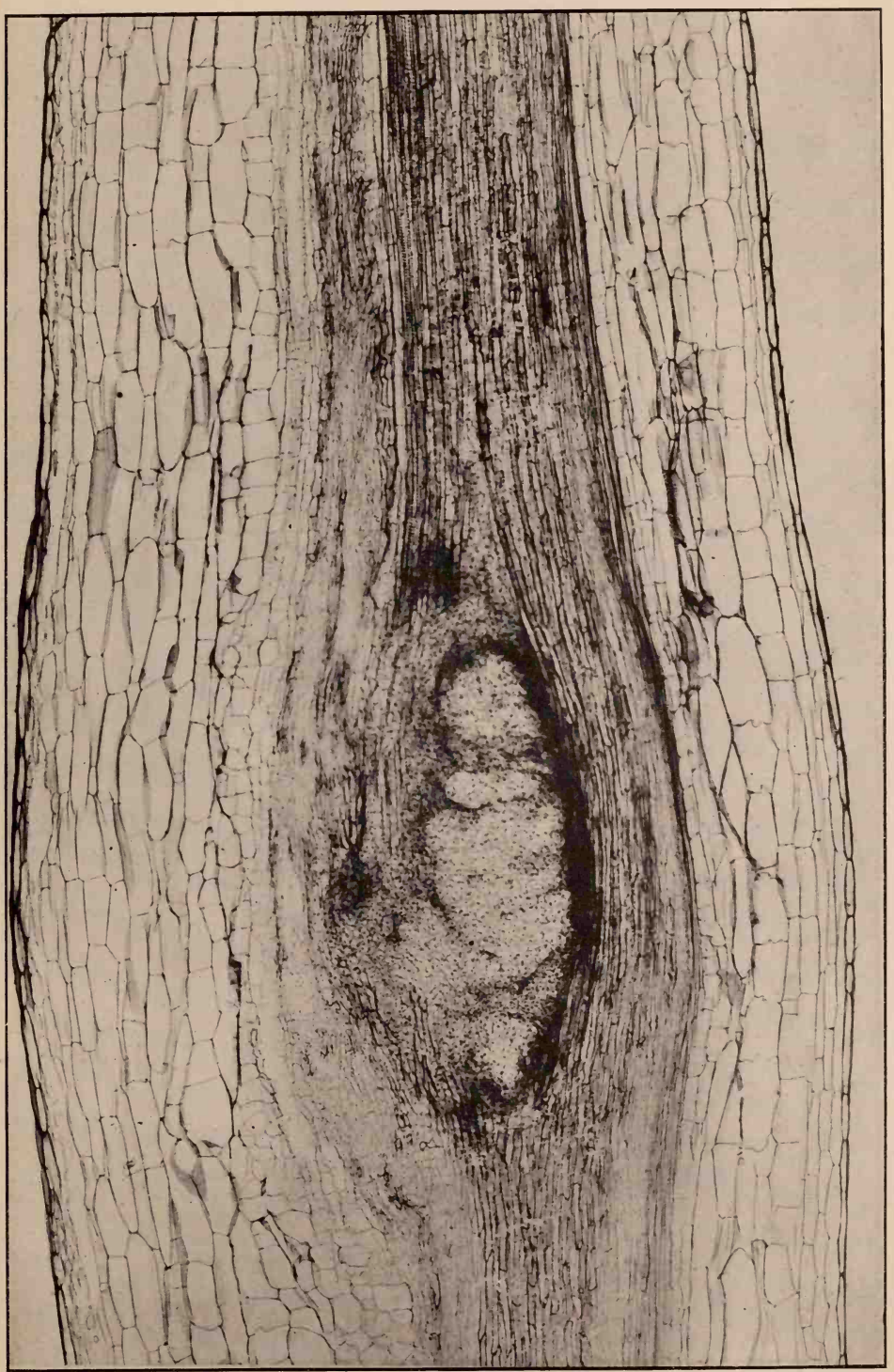
Daisy XIX. Cross section of base of petiole B in region of tumor-strand (p. 48).



Daisy XX. Primary stem tumor and secondary leaf tumors; also a cross section of stem at 1 (p. 48).

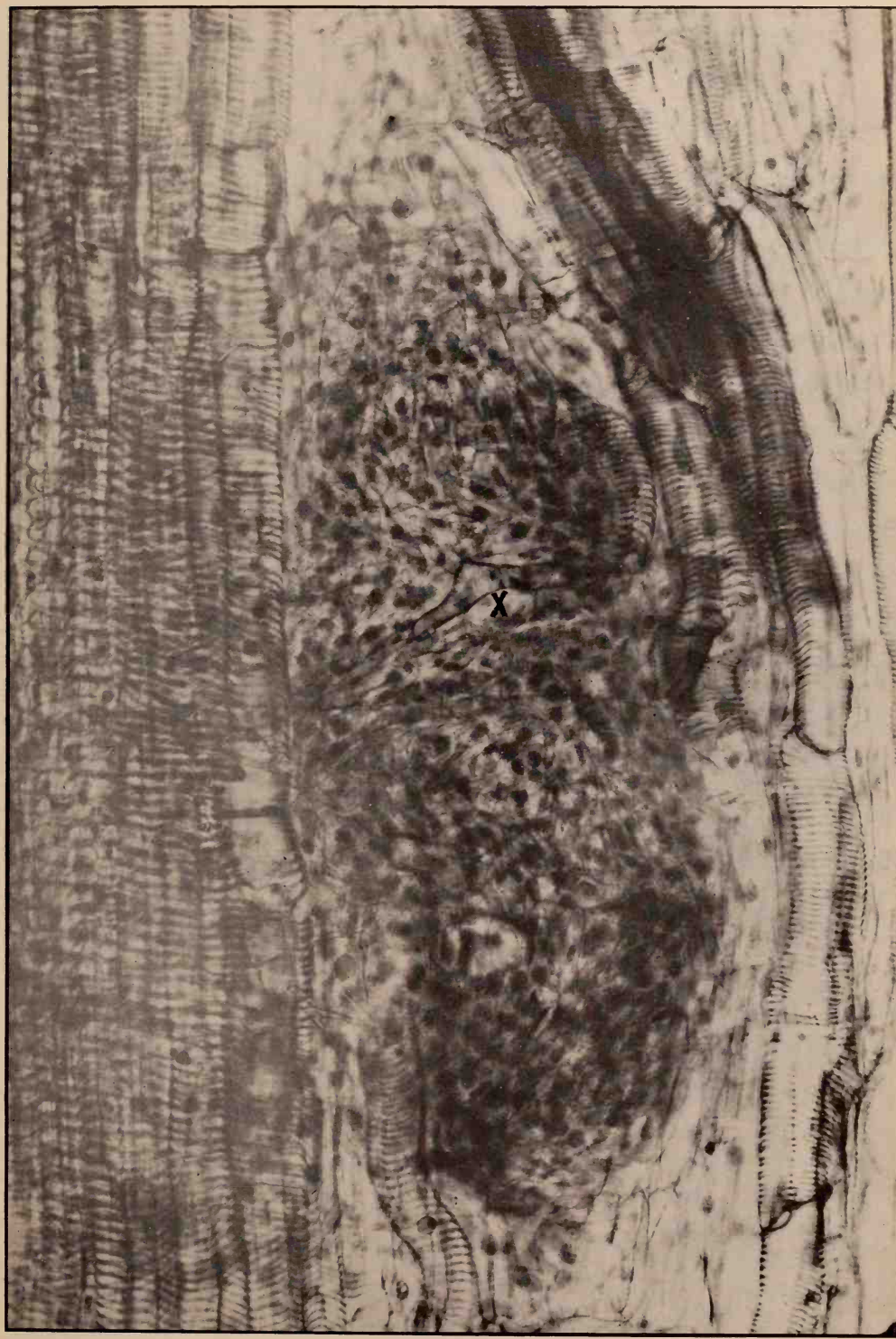


Daisy XX. Cross section of stem between tumors, showing small tumor-strand (p. 49).

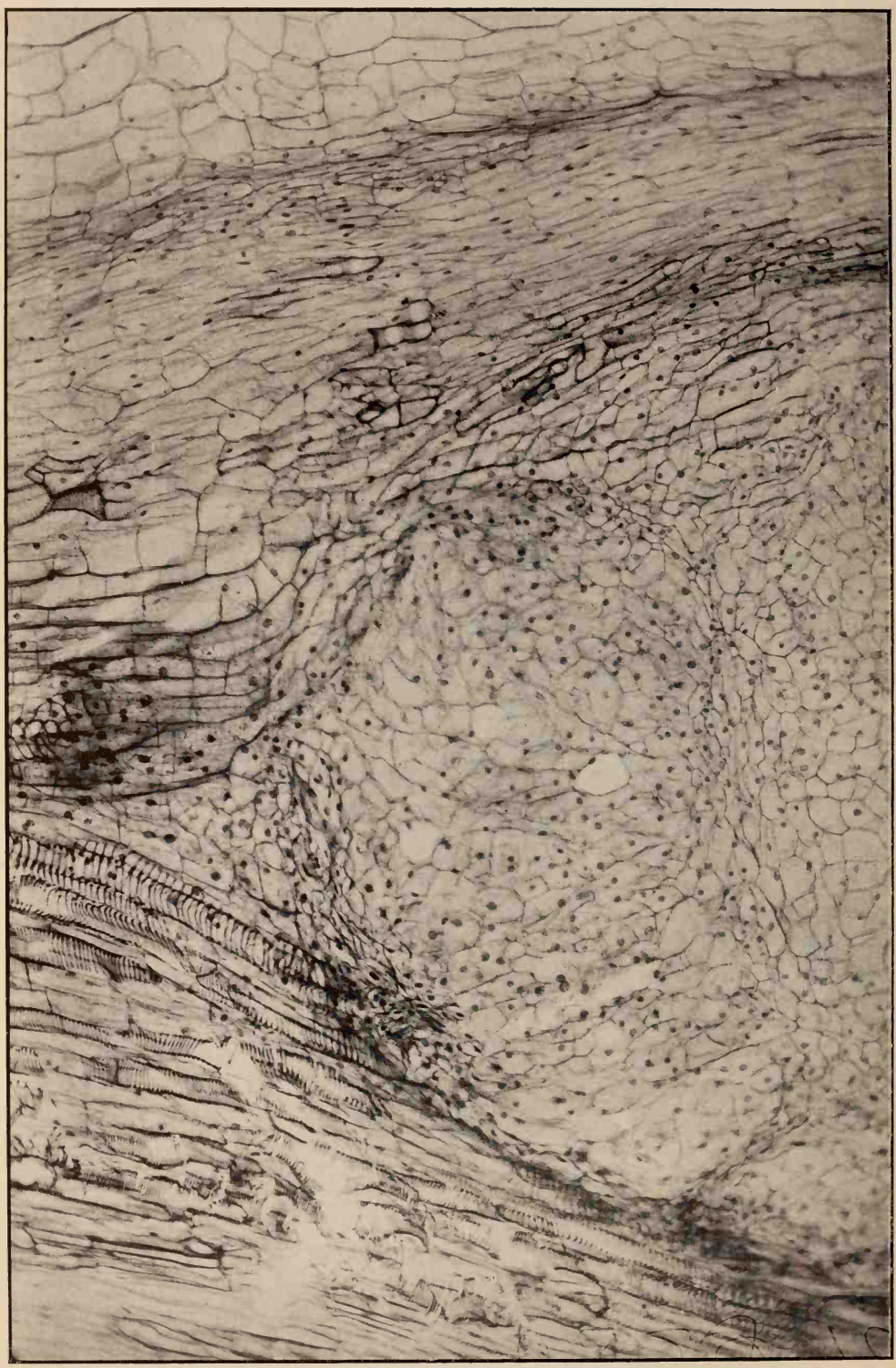


Daisy XX. Longitudinal section of petiole D, showing a young neoplasm (p. 49).

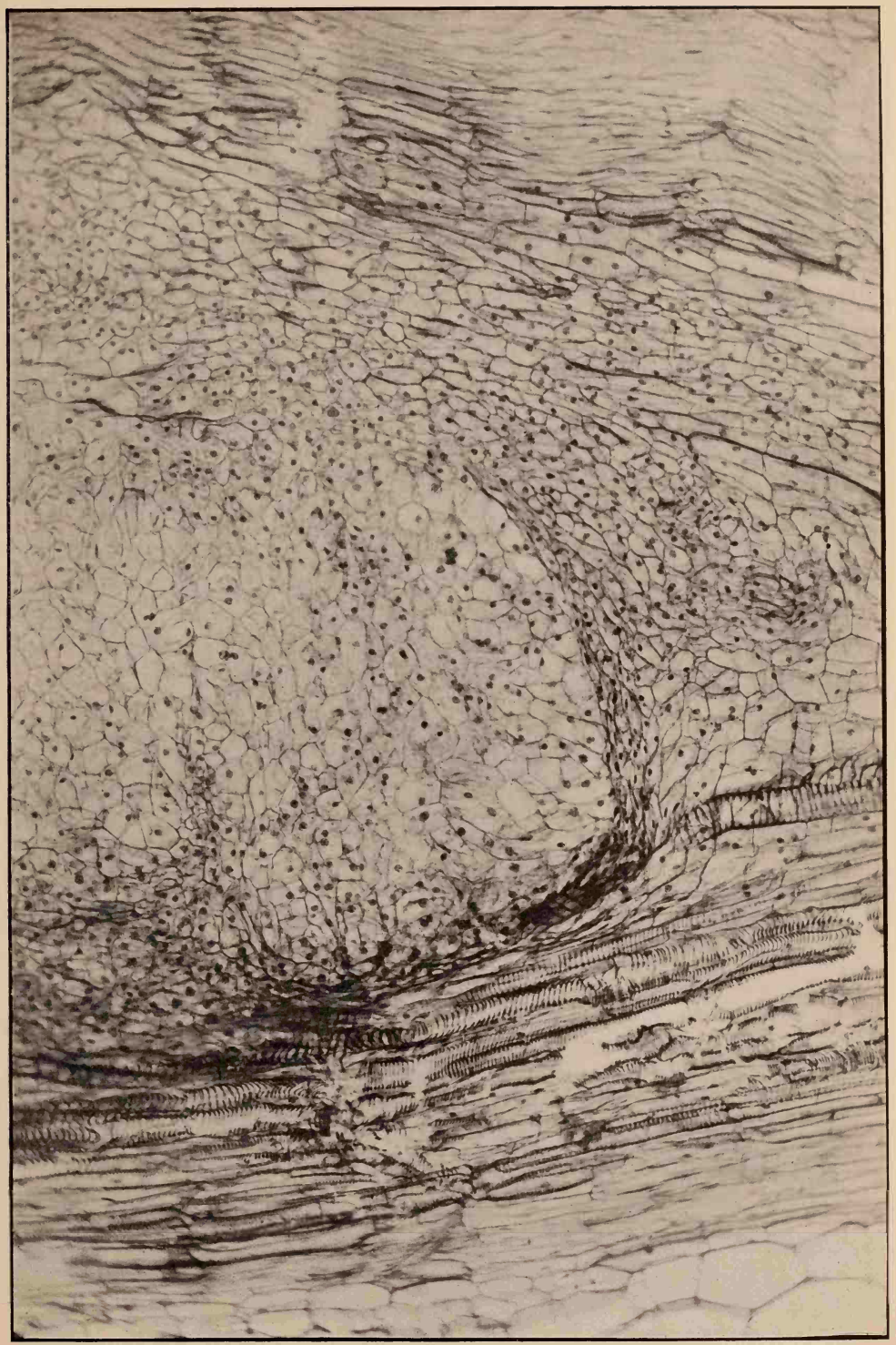
Daisy XX. Upper end of small neoplasm shown on Plate LXXXVIII (p. 49).



Daisy XX. Margin of small neoplasm, showing spirals wedged apart, and abnormal tracheids at X (p. 49).

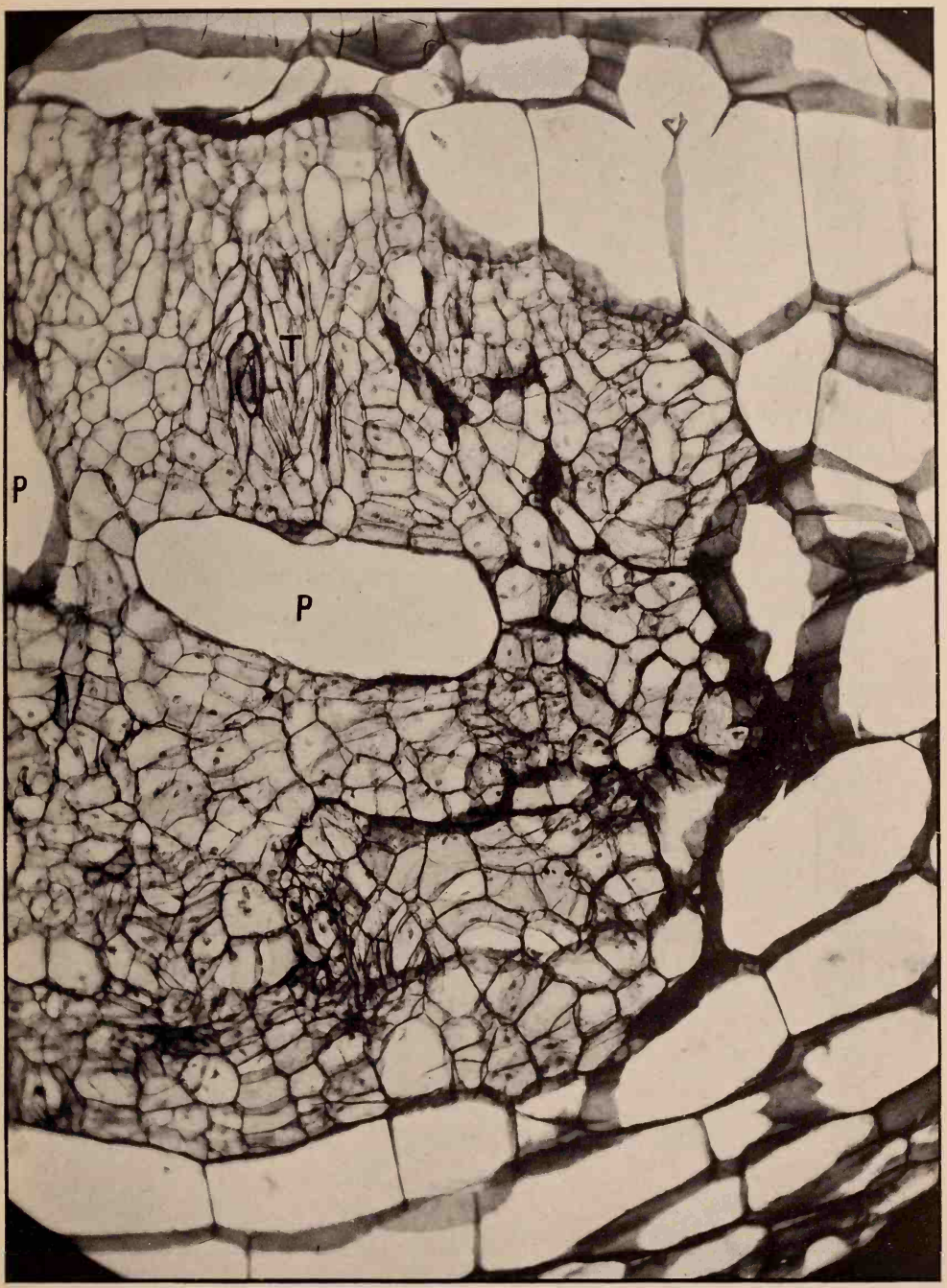


Daisy XX. One end of small unruptured tumor in petiole D (p. 49).



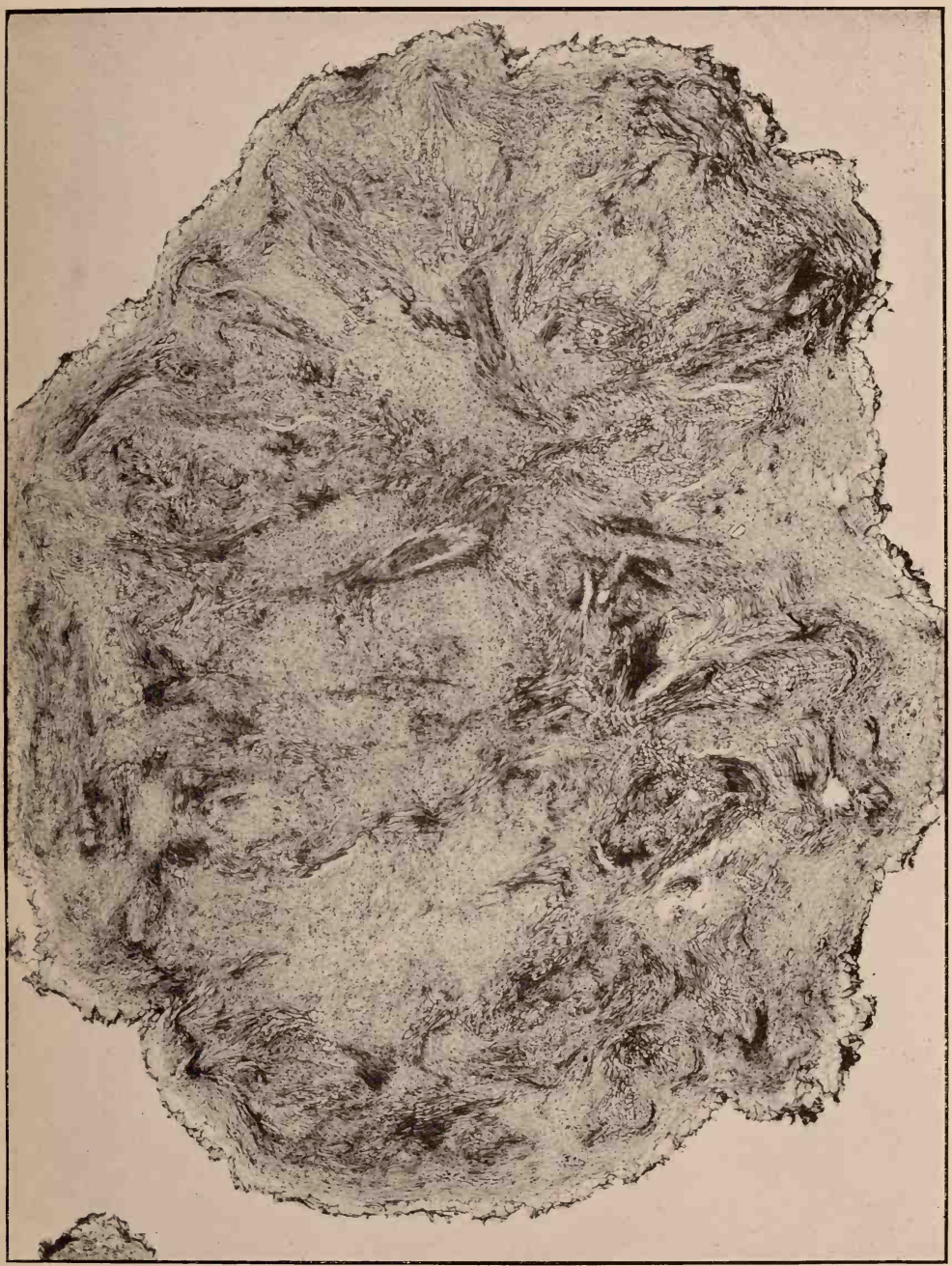
Daisy XX. Same as Plate XCI, but from the other end of the tumor (p. 49).

Daisy XX. Continuation of tumor-strand in petiole D (p. 50).

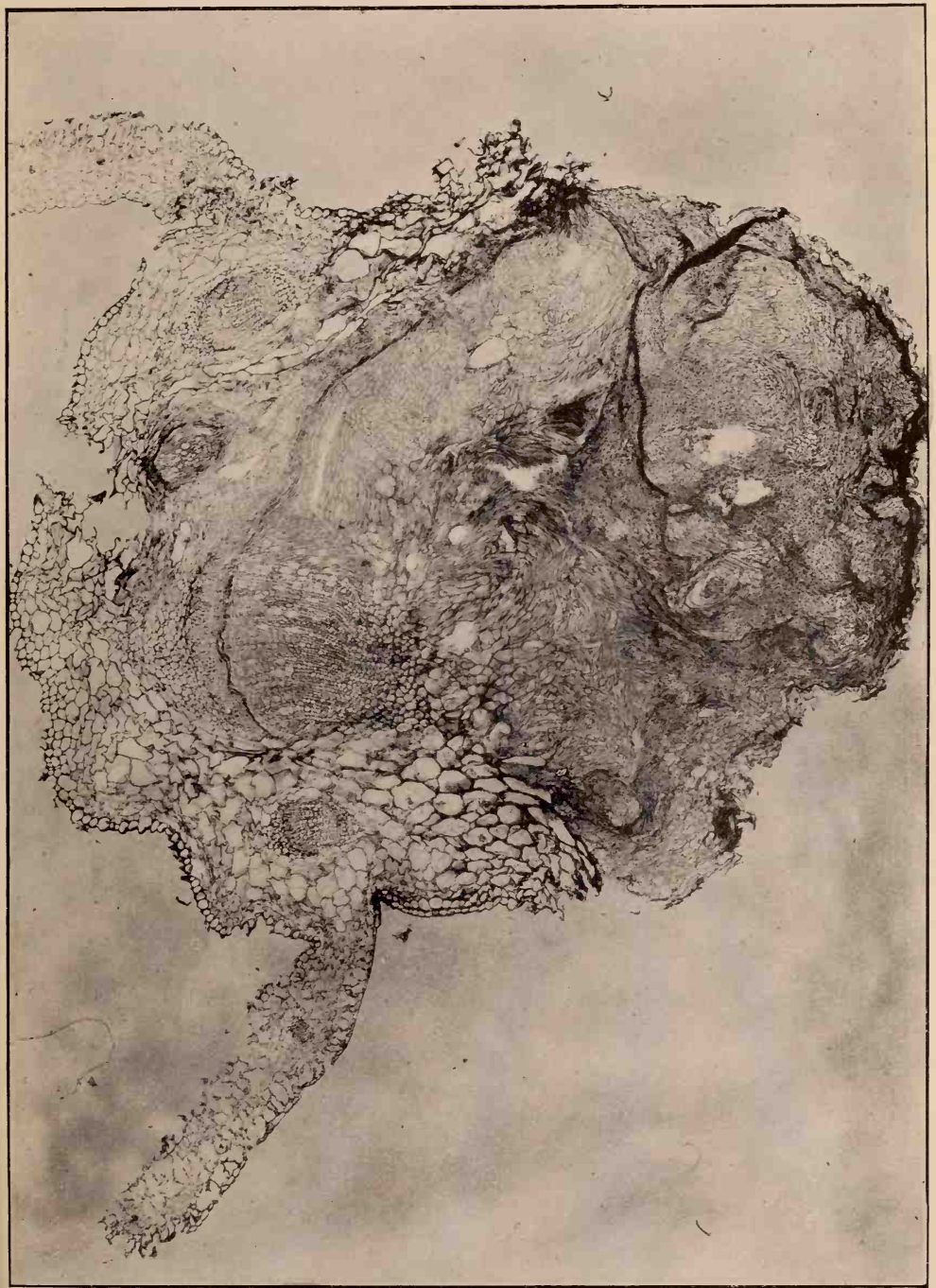


Daisy.—Margin of a tumor in a leaf stalk, showing tracheids at T, and inclusions of petiole parenchyma at P; at the right, ordinary ground tissue of the petiole (p. 50).

Daisy XXXI. Primary tumors on leaves (p. 50) resulting from single needle-prick inoculations introducing Bacterium tumefaciens. These galls do not have stem structure. For details, see next plates.

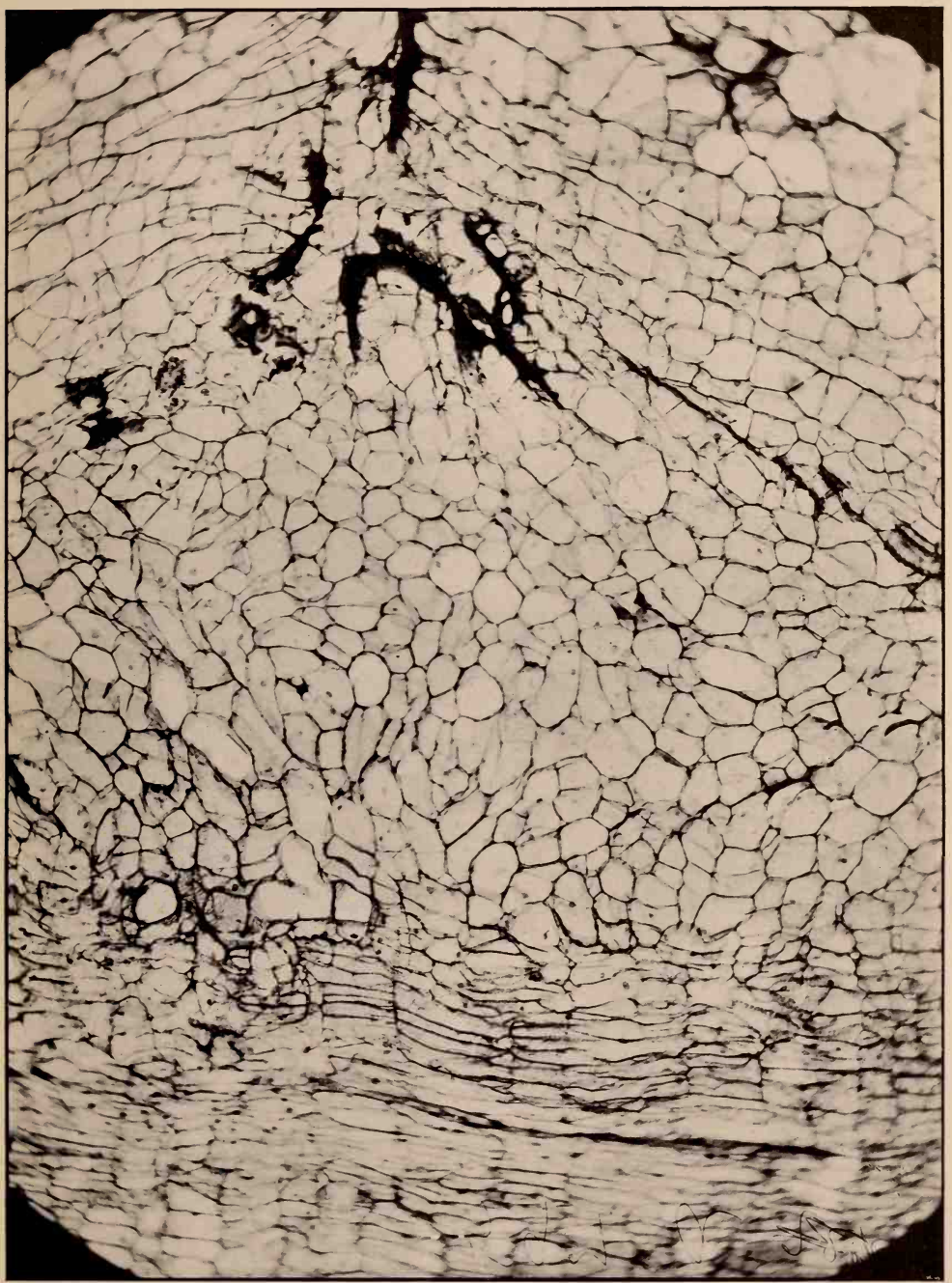


Daisy XXXI. Cross section of a primary leaf-tumor, for comparison with any of the secondary leaf-tumors, e. g., Plate LV. The numerous minute dots are deep-staining nuclei (p. 50).

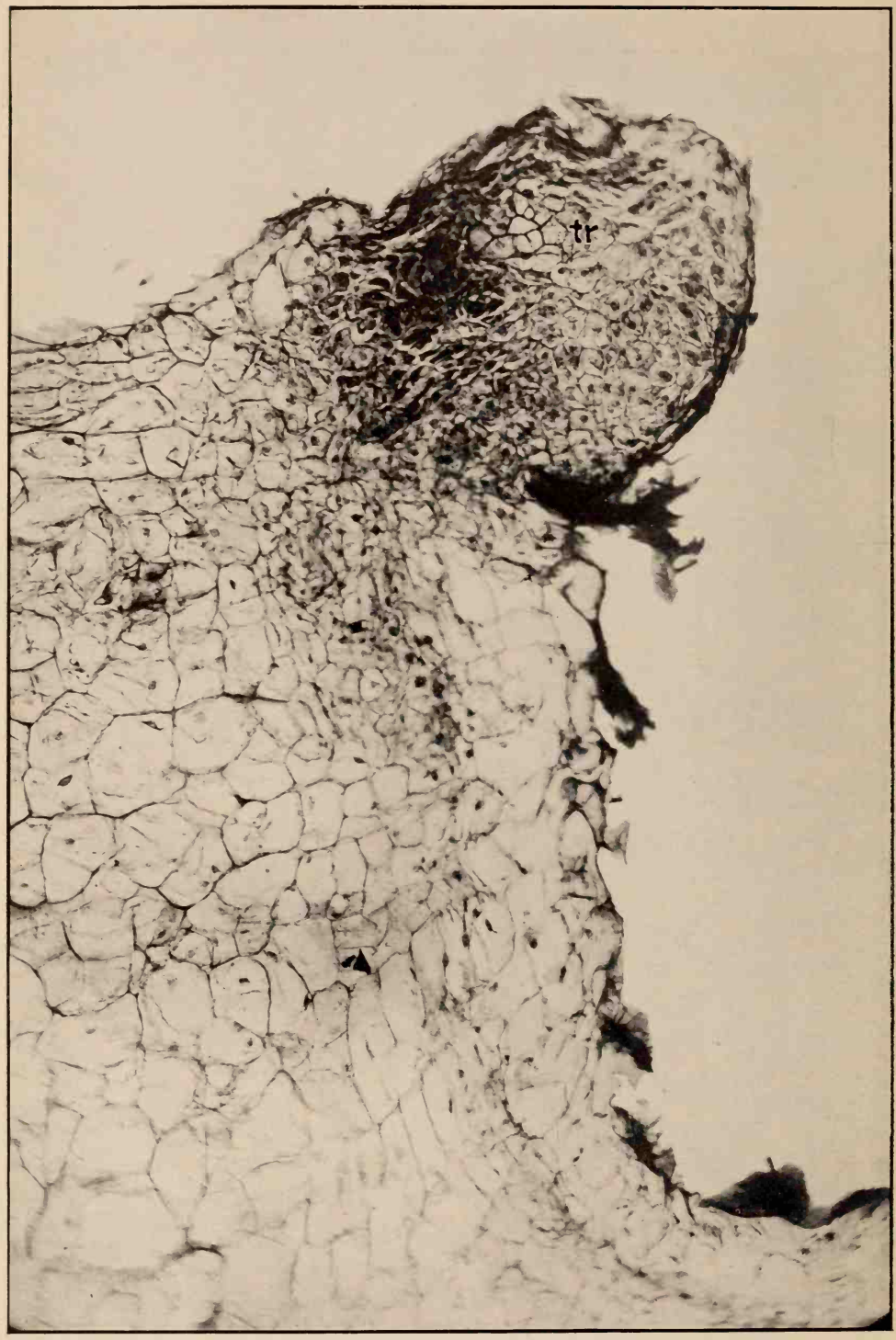


Daisy XXXI. Vertical section of another primary leaf tumor; normal part at the left (p. 50).

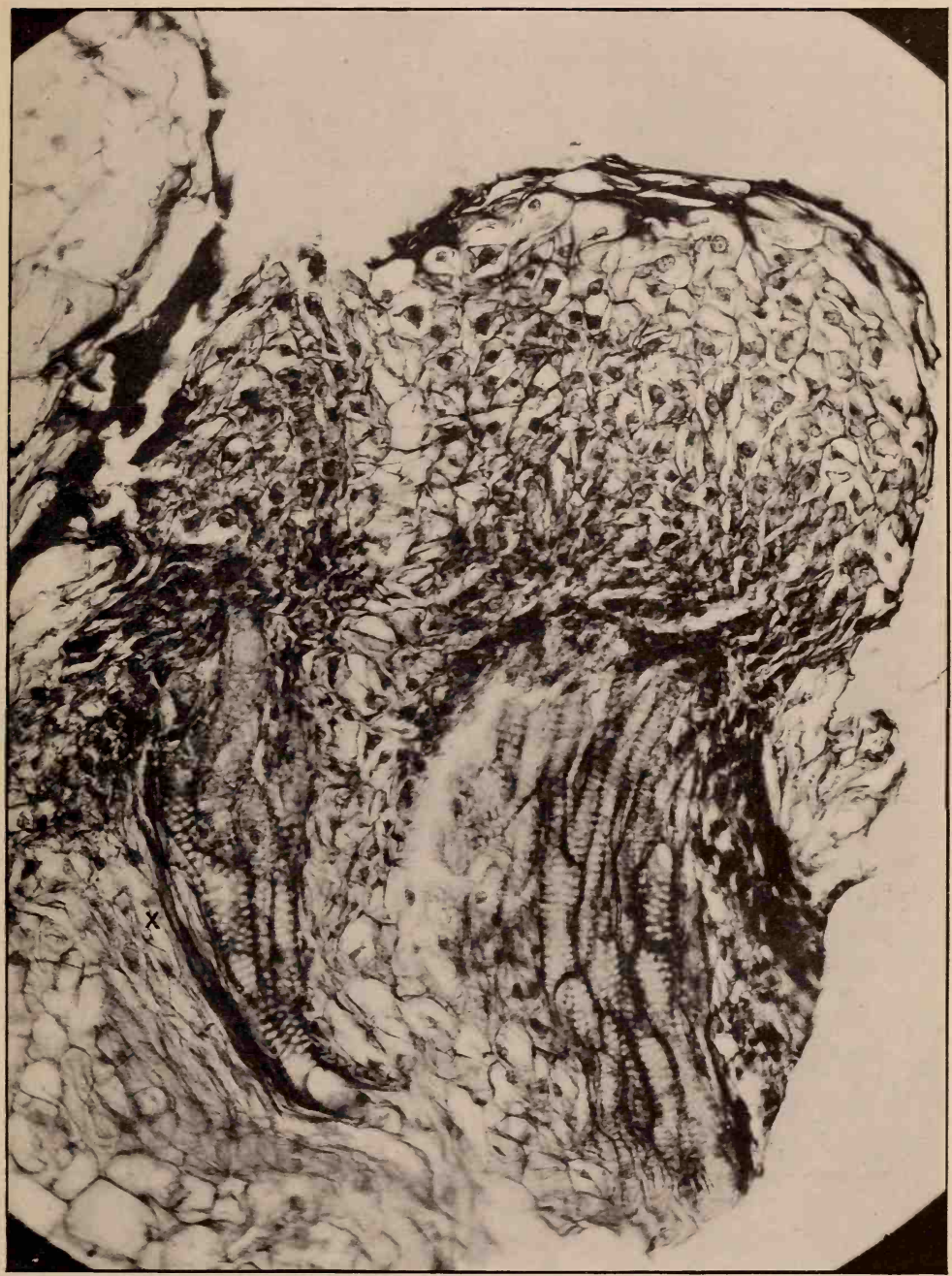
Tobacco. Infected needle-track through pith; no proliferation (p. 50).



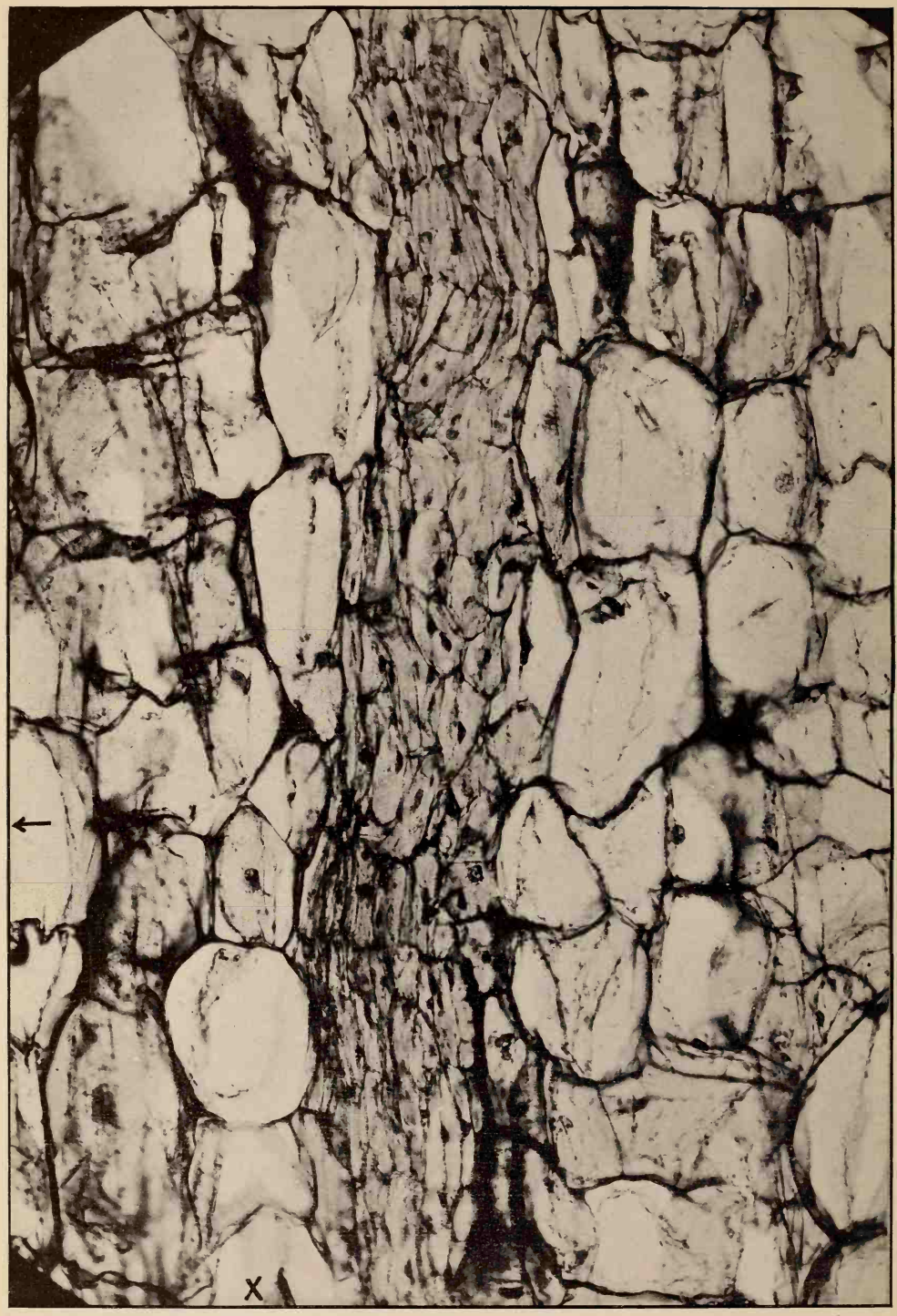
Tobacco. Longitudinal radial section, showing infected needle-track in cambium region with cells proliferating (p. 51). Time, 3 weeks.



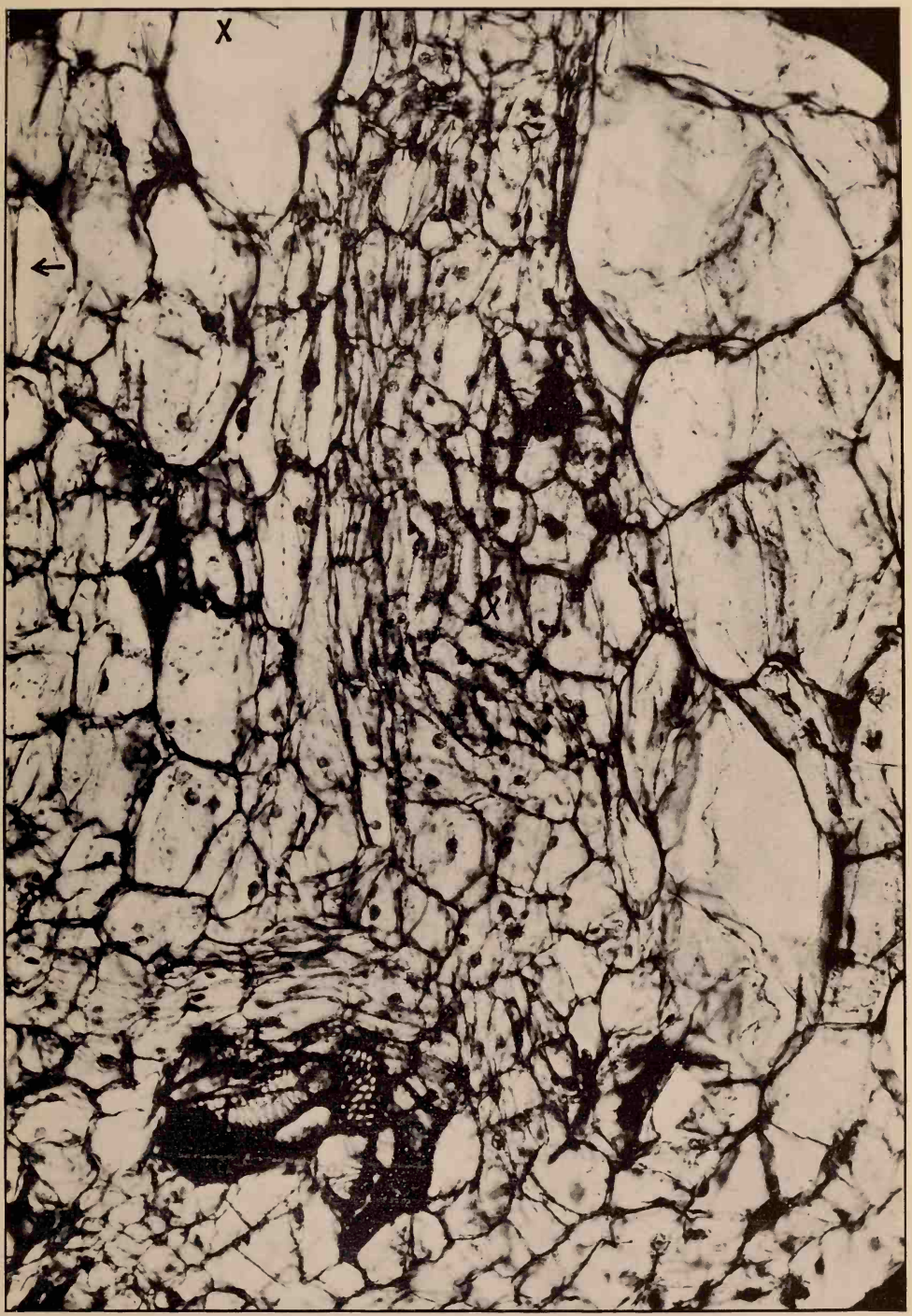
Tobacco. Margin of infected needle wound, showing tumor at top (p. 51).



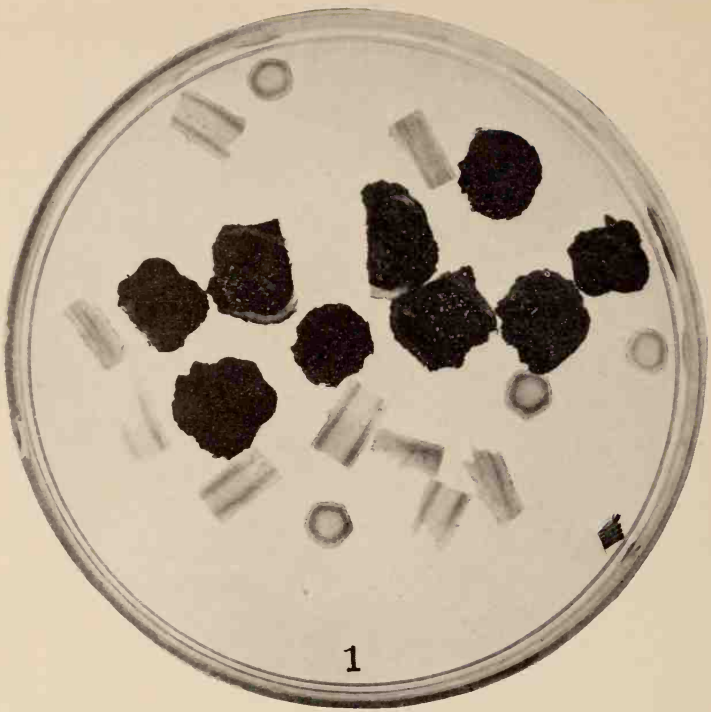
Tobacco. Margin of infected needle wound. Tumor in middle part of bark parenchyma; sieve tubes at X (p. 51).

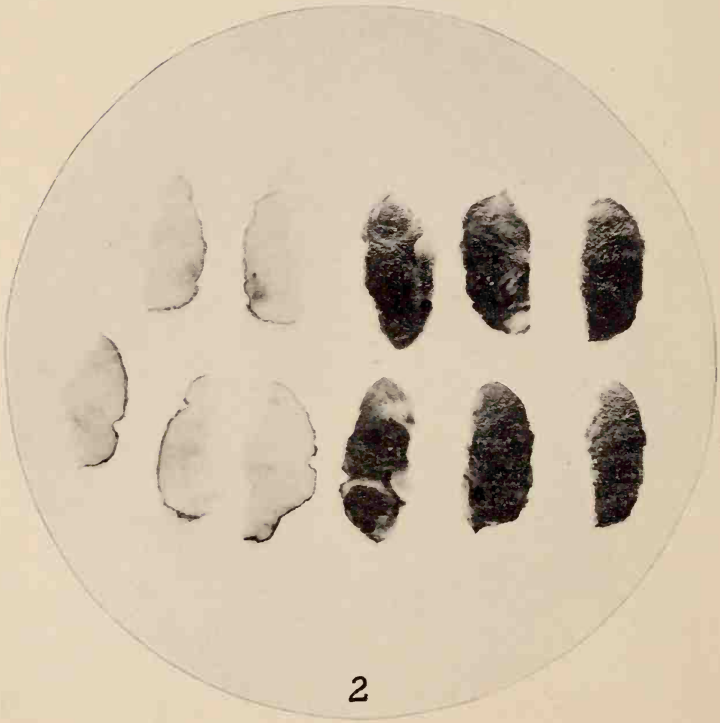


Tobacco. Tumor-strand in cortical parenchyma. Bottom joins top of next plate (p. 51).

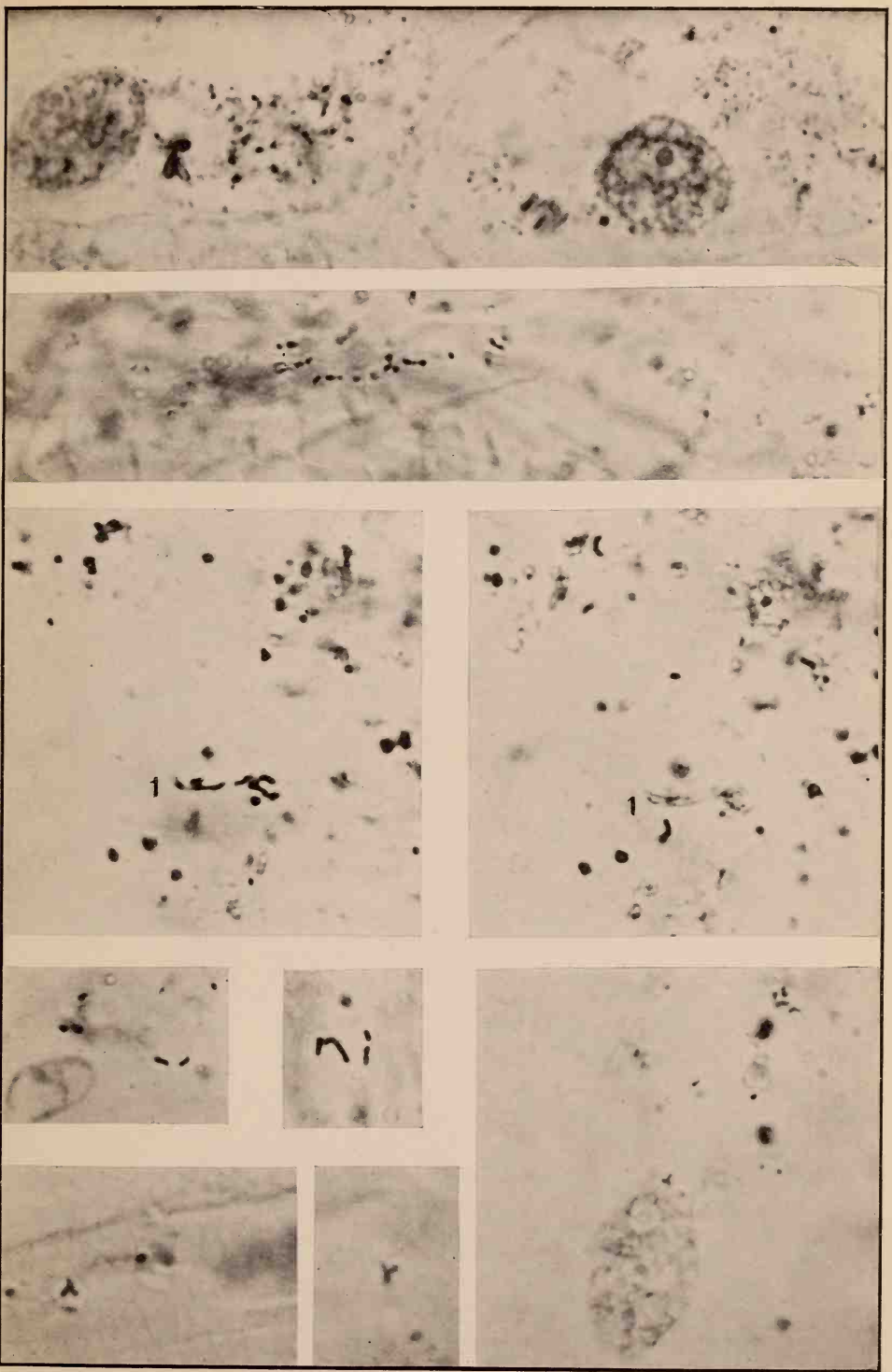


Tobacco. Tumor-strand with tracheids in cortical parenchyma (p. 51).

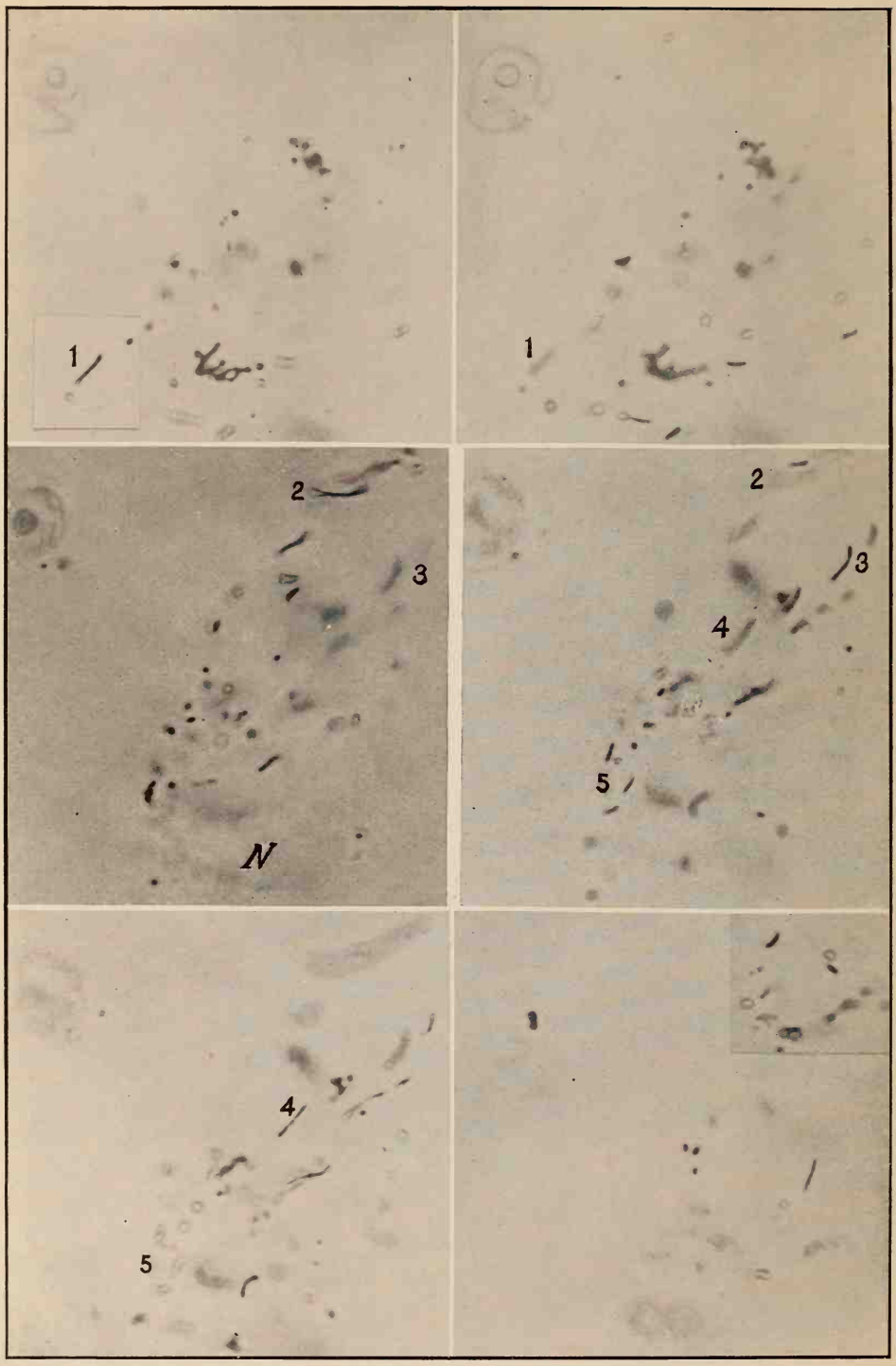




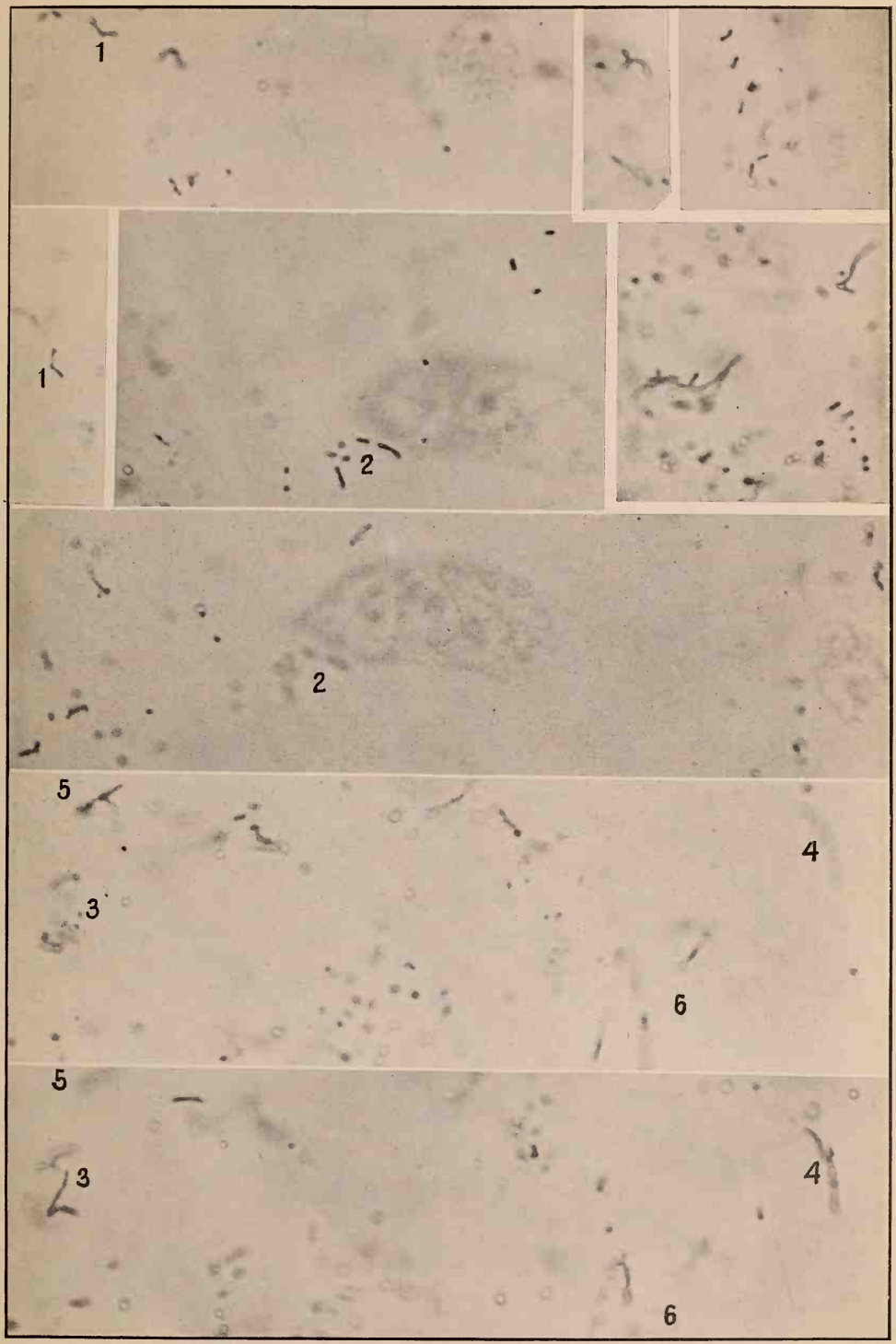
Daisy tumor. Reaction with chromium salts (pp. 23 and 51).



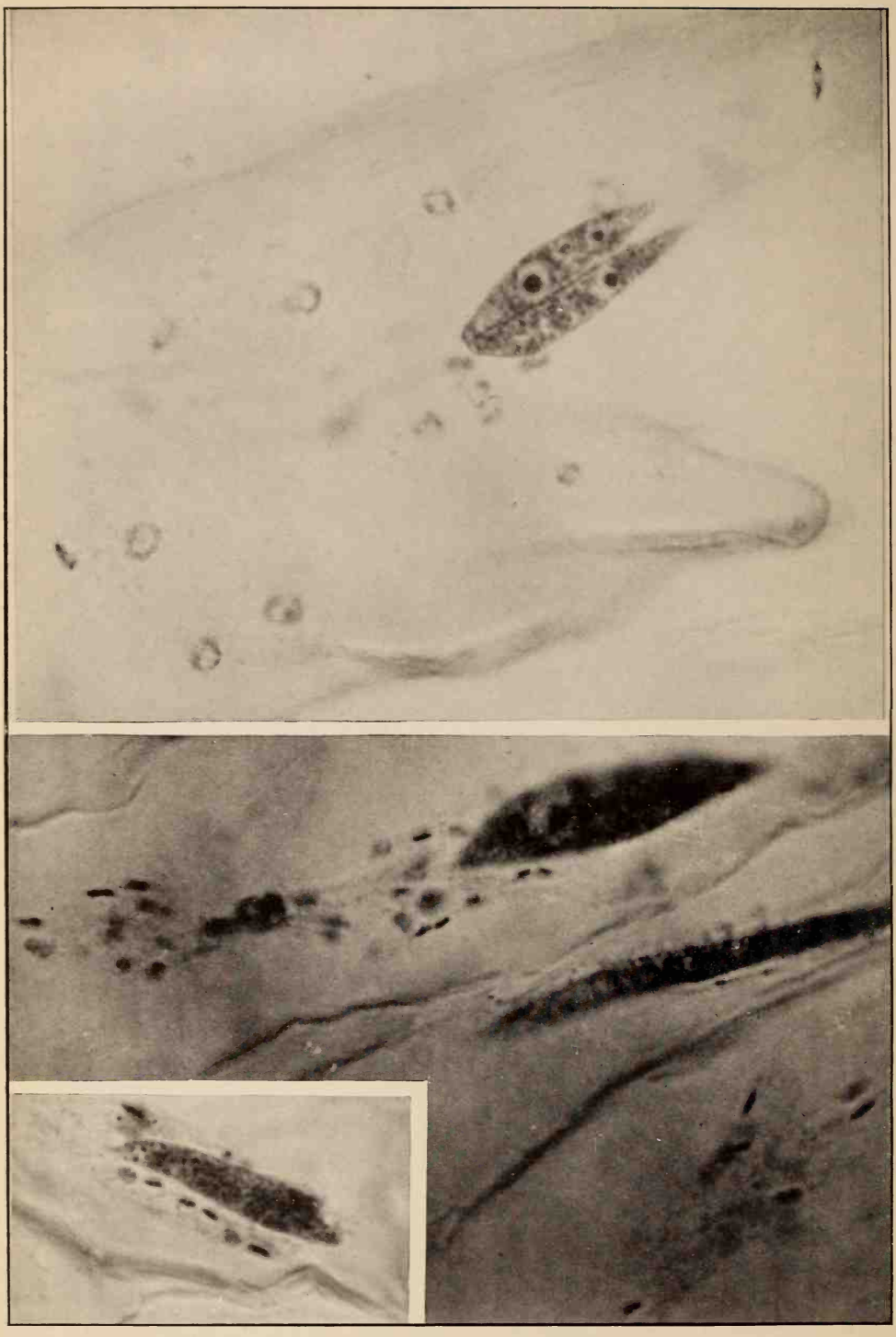
Daisy tumor. Gold-chloride impregnations (p. 52), showing bacterial rods and Y-bodies within the cells. X 2000.



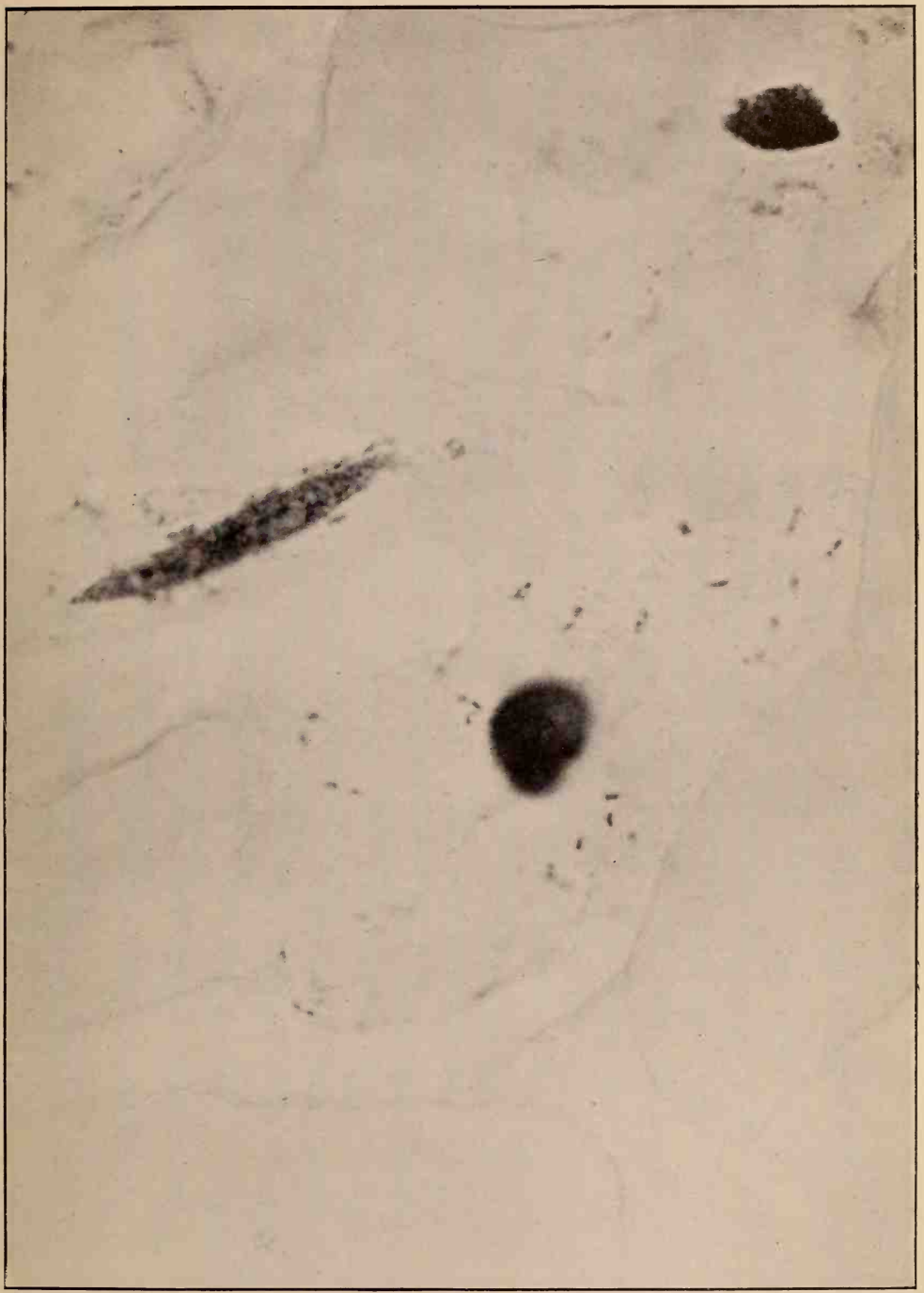
Daisy tumor. Gold-chloride impregnations (pp. 18, 19, 52), showing eight levels in a cell containing bacterial rods. X 2000.



Daisy tumor. Gold-chloride impregnations (p. 52), showing bacterial rods and Y's in various cells. X 2000.



Daisy tumor. Amyl Gram stain. Nuclei and chloroplasts which, when seen edge on, resemble bacteria (pp. 19 and 53). Upper nucleus dividing amitotically. X 2000.



Daisy tumor. Amyl Gram stain over washed, showing nuclei and rod-shaped bodies believed to be normal constituents of the cell (p. 53). The lower nucleus out of focus in order to make the other bodies distinct. X 2000.